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NUTRITIONAL DERMATOSES IN THE RAT

XII. The Influence of Deficiencies on the Extent of Injury and Healing Time of Liquid Mustard Gas Burns[1]

MAURICE SULLIVAN

Received for publication July 7, 1947

The mechanism involved in the injurious action of mustard gas is not known. It is believed that its vesicant and necrotizing properties may result from a combination of the mustard gas molecule with cellular tissue which includes some protoplasmic protein vital to cell life and perhaps a portion of one of the enzyme systems (1). In order to collect data which may prove to be of value in the eventual elucidation of the biologic effects of mustard gas, it was considered desirable to investigate the effect of nutritional deficiencies on the extent and healing time of cutaneous injuries produced by the gas. The rat was chosen as the experimental animal for the investigation, as this species is easily susceptible to a miscellany of deficiencies manifested by cutaneous changes (2). By utilizing the experimental nutritional dermatoses of the rat, it was possible to evaluate the influence of single and combined deficiencies and to make comparative observations of the effects of chemical damage to normal skins and to skins variously altered by atrophy, hypertrophy, edema, hyperkeratosis, crusting, vascular dilatation and ulceration.

THE EFFECT OF LIQUID MUSTARD GAS ON NORMAL HUMAN SKIN

Contact with liquid mustard gas always results in vesication and necrosis unless the amount applied is extremely minute. Only a small per cent of the gas is absorbed by the skin. The greatest portion of the absorbed gas becomes fixed in the epidermis. The remainder, which is free, is transmitted via the circulation to other organs.

[1] From the Department of Medicine (Dermatology) and the Department of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins University. Work Performed Under Contract OEM-CMR 82, Johns Hopkins University, Department of Medicine (Dermatology) for the Committee on the Treatment of Gas Casualties.

The cutaneous manifestations appear hours to days after contamination depending on the amount and state of the gas, the atmospheric conditions, and the sensitivity of the victim. Latent periods of two to six days have been noted after mild exposures (1). The extent of injury is always larger than the area contaminated. The peripheral area of erythema surrounding a central blister or central area of necrosis may have a diameter of one and a half to two times that of the skin area to which the vesicant was applied. Hypersensitivity to subsequent exposures frequently is established after cutaneous damage by mustard gas. Recrudescence, after apparent healing, sometimes occurs and is displayed as multiple vesicles on the periphery of the healing injury.

Davis (1) described the microscopic findings produced by mustard gas as follows: "There is a vacuolar or hydropic disintegration of the cells with eventual rupture of the cell membrane. Spaces formed by the progressive liquefaction of these cells become filled with fluid exudate to produce an intradermal vesicle. The base of the vesicle, which rests on the surface of the corium, presents an occasional remaining basal cell. The roof of the vesicle consists of an upper layer of epidermal cells that remain viable for a time and a lower layer of necrotic or disintegrating cells. The vesicle contains a homogeneous or fibrillar eosinophilic staining fluid in which there are a few polymorphonuclear leukocytes. Some of the hair follicles appear necrotic for a short distance from the surface, but there are no significant changes in the sebaceous glands, sweat ducts or sweat glands. There is some edema of the superficial corium with capillary dilatation and perivascular edema and mild perivascular infiltration of lymphocytes and polymorphonuclear cells." The description of Davis presumably applies to the conscious experimental contamination of the skin with small measured amounts of liquid mustard gas, as contamination with large amounts produces necrosis and ulceration of severe degree.

THE EFFECTS OF LIQUID MUSTARD GAS ON NORMAL RAT SKIN

Contact of liquid mustard gas with rat skin produces necrosis but never results in macroscopic vesiculation. The failure of mustard gas to react with gross vesiculation in rat skin, and in the skins of other small fur bearing animals, has been a subject of much speculation and

has prejudiced some investigators against the use of experimental animals for procedures calculated to measure the injurious cutaneous effects of the agent. The blistering or vesicant phenomenon is rigidly associated with the notion of "a vesicant", and considerable quantitative significance is attached by some observers to the sizes of experimentally produced vesicles and bullae. Such strict interpretation of quantitative blister significance does not hold when the gross effects and histopathology of mustard gas injuries are appreciated and broadly viewed. In the case of contamination with large amounts of liquid mustard gas vesiculation occurs only on the periphery of the injured area, the central portion of which is necrotic and dry. In such a circumstance, quantitative significance of blister area is obviously untenable. The daily and sometimes hourly increase in the size of vesicles and bullae due to the spreading effect through the skin and the recrudescence of vesicles in healing areas also preclude the strict quantitative value of vesiculation. Individual variations due to many factors, including hypersensitivity and increasing states of sensitivity, are additional arguments against the necessity for producing blisters when quantitative effect is the consideration. Failure to produce the manifestation of gross vesiculation in the rat was viewed as no disadvantage in this study, as it was found that it was possible to produce other measurable signs of cutaneous damage.

Fifteen hundred applications of Levenstein 95 per cent pure liquid mustard gas were made to the frontal, parietal and occipital regions of the scalp, the sides of the snout, the anterior and posterior surfaces of the ears, the necks, the shoulders, the backs, the flanks, the abdomens, the dorsal surfaces of the paws, the heels, the plantae, and the tails of 150 adult McCollum stock rats. Nembutal anesthesia was employed. The hair was shaved from the test areas with electric clippers immediately before each application. Small amounts of 180 to 350 gammas were applied with a cylindrical stainless steel rod having an applicating surface of 6.5 mm. Larger amounts of 1 to 5 milligrams were applied with a specially constructed microsyringe. It was found that the most suitable sites for testing were the parietal regions of the scalp, the posterior aspect of the neck, the back, flank, and abdomen. In the anesthetized rats these locations are plane surfaces. They are also convenient locations for measurements and

observations. In these sites it was possible to afflict standard reproducible injuries which were remarkably uniform. The pattern of the injuries was similar for the different amounts applied. The extent of each injury was proportional to the amount of the vesicant applied. The glistening liquid remained on the surface for only a few seconds after the applications. Three to four hours later there was blanching in and immediately around the site of contamination. Twenty four to forty eight hours later there was a round or oval plaque in the center of which there was a pale area; as a rule the central area of paling constituted one-half of the plaque. In some cases the entire plaque was erythematous and edema extended to the periphery, resulting in a slightly raised erythematous plaque not unlike a flat topped or slightly convex urticarial wheal. In some of the severe reactions, there was a greater intensity of erythema; in a few instances punctate hemorrhage occurred. The first stage of erythema and/or edema lasted for 2 or 3 days. Serous exudate was then observed in the central portion within an erythematous halo that persisted for 5 or 6 days. Seven or 8 days after the application of mustard gas the halo of erythema subsided and was replaced by another halo which spread centrifugally and in which the surface product of normal sebaceous secretion was absent. The peripheral bands were noted particularly on the backs where a thin deposition of sebaceous material is present normally in adult rats. Crusting increased in thickness. When the crust was forcibly detached, there was an underlying superficial ulcer. The stage of crusting lasted 10 to 14 days, following which there was healing with superficial scar formation. During healing there was alopecia in the scarred area, but on the periphery of the scar there was often increased growth of hair. There was a gradual restitution of the formation and deposition of the brown sebaceous material, and the pale halo surrounding the scar was not evident 6 to 8 weeks after the injury. In pigmented areas the scars were depigmented.

For the purpose of making quantitative comparisons it was necessary to divide the visible signs into 2 categories, namely, those which could be conveniently measured in millimeters or centimenters and those which had qualitative significance only. In the first or "measurable" group were erythema, central pallor, ulceration, crusting,

failure of sebaceous secretion, and scarring, the diameters of all of which could be recorded. In the second, or "immeasurable" group, were such signs as intensity of erythema, punctate hemorrhage, depth and thickness of edema, crusts, ulcers, and infiltration. Although the latter group of signs contributed to the comparative qualitative evaluation of injuries, the former group of signs served principally as a basis for comparison in the deficient and control groups because it was possible to ascribe to them numerical values.

Microscopic examinations of skin biopsies taken 15 minutes, 30 minutes, 1 hour, 5 hours, 8 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 8 days, 15 days, 21 days, 22 days and 30 days after the applications revealed changes which accounted for the gross signs of injury. These changes were studied and recorded and compared later with the changes observed in the injuries of deficient rats and their normal controls. Fifteen minutes after application of liquid mustard gas there are no discernible changes in the epidermis. In the cutis there are mild diffuse edema, sparse cellular infiltration, and a few dilated and hyperemic blood vessels. At one hour some of the epidermal cells are shrunken and distorted. In the cutis there are edema, mild diffuse cellular infiltration and dilated hyperemic vessels. The walls of some of the vessels appear to be damaged. At 5 hours there is an increase in the number of distorted epidermal cells; a few are vacuolated. Throughout the cutis are diffuse cellular infiltration, edema and hyperemia. At 8 hours the cellular infiltration extends deeper into the cutis. At 24 hours there are numerous distorted, flattened, vacuolated cells in the epidermis. There is acantholysis of the basal layer, and immediately beneath the epidermis are patchy areas of edema. The cells of the sebaceous glands and hair follicles are distorted; some are shrunken and some are dilated and disintegrated. There is an increasing amount of edema in the lower half of the cutis. At 48 hours there are several well defined areas of vesiculation in the epidermis, although grossly vesiculation is undetectable. Numerous areas of the epidermis are invaded by exudative cells and the structure of the epidermis is no longer recognizable. Many of the hair follicles and sebaceous glands are infiltrated by cells. In the cutis there is still diffuse cellular infiltration, hyperemia and edema. At 3 days there is separation of the epidermis from the

cutis and crusting on the surface. Some of the sebaceous glands are still intact but the majority have been destroyed. There is some edema and cellular infiltration throughout the cutis, most extensive in the lower half. The connective tissue shows some fragmentation. The underlying muscle is undisturbed. At 8 days a thick crust replaces the destroyed epidermis. Throughout the cutis there is extensive cellular infiltration which is most prominent in the perifollicular regions. In the lower cutis there are dilated hyperemic vessels and edema. At 30 days there is restitution of the epidermis, but in the injured area the epidermal cells are flattened and distorted, and there is destruction of the hair follicles in the central area. New connective tissue occupies the upper portion of the cutis.

When liquid mustard is applied to or accidentally comes into contact with human skin hypersensitivity to subsequent exposures frequently occurs. It was important to determine whether a state of hypersensitivity could be produced in rats and to know whether hypersensitivity and varying degrees of sensitivity should be taken into consideration in evaluating results of mustard gas injuries in rats.

Prior to the report of Kenton (3) in 1941, the rat was considered an unsuitable animal in which to produce phenomena of hypersensitivity. Numerous attempts to sensitize rats to egg white have failed (4). Several investigators have recorded their failures to sensitize rats to other substances (5). Kenton (3) showed that the rat responded to repeated antigenic stimuli with only slight antibody production and that it was not possible to demonstrate the Arthus phenomenon in the skin of actively immunized rats. However, when large amounts of a potent immune serum were administered to rats by passive transfer the Arthus phenomenon occurred regularly upon injection of the specific antigen. According to Kenton, these observations indicated that "the tissue of the rat will respond with typical inflammatory reaction whenever antigen and antibody are present in sufficient concentration".

Experiments 1 and 2, described below, demonstrate that: a. It is possible to produce in rats a phenomenon of hypersensitivity to liquid mustard gas by a method of multiple applications in which mustard gas is reapplied to sites previously injured by mustard gas. b. The phenomenon of hypersensitization may be avoided if each of

the multiple applications is made on a skin site previously undamaged by mustard gas.

Experiment 1: Thirty-three adult McCollum stock rats were divided into group A and group B. During the 5 months' period of the experiment both groups were reared under identical environmental and dietary conditions.

To the 14 rats in group A applications were made in a manner calculated to produce hypersensitization. With a rod having an applicating diameter of 6.25 mm., 3 equidistant applications were made on the right side of the back of each rat. Approximately 1 week later 3 similar equidistant applications were made on the left side of the back of each rat. Again, approximately 1 week later, 3 similar equidistant applications were made on the mid line of the back of each rat. After stages of erythema, edema, ulceration and crust formation, each injured area healed by scarring. At 3 weeks, 5 weeks and 7 weeks after the initial applications, mustard gas was reapplied respectively to one of the 3 linear groups of 3 scars. At each of the previously injured areas where the mustard gas was reapplied, there was repetition of the stages of injury previously observed, and eventually there was healing with more extensive scar formation than that which followed the initial injuries. On 15 occasions an interesting phenomenon was observed 24 hours after the reapplication of mustard gas to 3 previously injured sites (scars) in one row. In and circumjacent to the 3 scars in one of the other rows erythema and edema were remarked. The signs of reactivation subsided in 24 hours whereas the signs of inflammation at the sites of reapplication persisted and produced ulceration and scarring. Reactivation in the scarred areas was thus accomplished by reapplying mustard gas to other previously injured (scarred) sites. Twelve weeks after the initial applications of mustard gas, single applications were made with the same rod at sites that were not previously injured. There was no evidence of reactivation in the previously injured areas. The extent of the injury was equal to that which followed the initial applications.

Experiment 2: To each of the 19 rats in group B applications were made with 8 smaller, graded applicators. On the backs and abdomens 3 rows of 7 applications were made with applicators having diameters of 0.5, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5 mm. Reapplications were made in the manner described above (group A). The same phenomenon of reactivation was observed. Two months later numerous applications were made with a rod having a 6.25 mm. diameter to *previously uninjured* sites on the head, ears, neck, flanks, paws, and tail. Over a period of 4 months many such applications were made to uninjured areas and the previously injured (scarred) sites were carefully avoided. There was no evidence of the reactivation phenomenon in the 4 months' period of observation. During the 4 months' period the average extent of injury at every site equalled the average extent of injury observed in rats to which only single applications had been made.

In all subsequent experiments care was exercised to avoid the application of the

vesicant to previously injured sites and the consideration of individual hypersensitivity was disregarded in evaluating results.

Observations of the extent of injury and healing time were made in groups of rats conditioned by deficiencies of carbohydrate, protein, fat, riboflavin, pyridoxine, pantothenic acid, biotin, vitamin A, and magnesium, and deficiency of the vitamin B complex other than thiamine. Cystine and choline, para-amino benzamide and large doses of vitamin B complex were administered to rats to determine the influence of these agents on healing time. Following are summaries of the experiments:

CARBOHYDRATE DEFICIENCY

Experiment 3: A litter of 8 matched young rats was divided into 2 groups, A and B. To the control group A, the following diet was fed: sugar 54%, casein 20%, lard 10%, salts 6%, yeast 10%. The sugar content of group B was reduced from 54% to 24% and the percentages of casein and lard were increased so that the diet was composed of 24% sugar, 30% casein, 30% lard, 6% salts, and 10% yeast. Oleum percomorphum and alpha tocopherol supplied vitamins A, D, and E to both groups. The growth curves in the 2 groups were approximately equal during the next 3 weeks. A dose of 1.77 miligrams of liquid mustard gas was applied to the shaved back of each rat. There were no general ill effects. The rats were observed for 2 months and comparative measurements were made weekly. Rate of healing and extent of injury were approximately the same in each group.

Experiment 4: A litter of matched young rats was divided into 2 groups, C, consisting of 2 rats, and D, consisting of 6 rats. To the control group C, the following diet was fed: sugar 54%, casein 20%, lard 10%, salts 6%. The sugar content of the diet fed to group D was decreased from 54% to 24% and instead of increasing the percentages of casein and lard to make up the deficit, 30% agar-agar was added so that the ration consisted of 24% sugar, 20% casein, 10% lard, 6% salts, 10% yeast, and 30% agar-agar. Oleum percomorphum and alpha tocopherol supplied vitamins A, D, and E to each group. The weight increase in group D was considerably less than that in group C. The control rats weighed 106 and 132 grams and the deficient rats varied from 50 to 95 grams. To the shaved backs of the control rats, and to 4 of the rats in group D, doses of 1.77 milligrams of liquid mustard gas were applied. Two of the deficient rats died 5 days after the applications. The other 2 survived for 2 months and continued to gain weight comparable to the 2 deficient litter mates that were not burned. Leukocyte counts were made before each application and 3 days later. The leucopenia which followed was not more marked in the deficient rats than in the control rats. In the deficient rats that were burned, there developed transient signs of pantothenic acid deficiency and

abdominal distention. Healing time and extent of injury were approximately equal in each group.

Conclusion: Carbohydrate deficiency apparently had no influence on healing time and extent of injury due to liquid mustard gas, when the deficiency of carbohydrate in the diet was compensated for by an increase in protein and fat. When the deficit of carbohydrate in the diet was not replaced by protein and fat, and inanition resulted, there were signs of transient pantothenic acid deficiency in the burned rats, although the cutaneous signs of injury were not enhanced and healing was not prolonged.

SEVERE PROTEIN DEFICIENCY

Experiment 5: A litter of matched young rats was divided into group A, consisting of 2 rats, and group B, consisting of 4 rats. To the control group A the following diet was fed: casein 20%, lard 10%, sugar 54%, salts 6%, and yeast 10%. A low protein ration was fed to the rats in group B; the percentage of casein was reduced from 20% to 8% and the percentages of lard and sugar were increased to 16% and 60% respectively. Oleum percomorphum and alpha tocopherol supplied vitamins A, D, and E. During the first 2 weeks after the diet was started the weight gain in group B was less than that in group A. The doses of liquid mustard gas were calculated on a weight basis. To the shaved back of each rat .59 milligrams per 20 grams of body weight was applied with a microsyringe. The dose for the control group was 2.36 milligrams and that for the deficient group was 1.77 milligrams. Despite the fact that smaller doses of liquid mustard gas were applied to the deficient rats the extent of the injuries was greater and healing was delayed for three weeks (Figure 1).

The stage of deficiency in Experiment 5 was advanced and one not likely to be encountered in humans. Therefore, another experiment was performed in which adult rats were used in order to produce a less depleted condition in the animals.

PROTEIN DEFICIENCY OF MILD TO MODERATE DEGREE

Experiment 6: Forty-six adult rats ranging in body weight from 195 to 235 grams were divided into 2 groups, C and D. To the control group C consisting of 12 animals was fed the following diet: casein 20%, sugar 54%, lard 10%, yeast 10%, salts 6%. Oleum percomorphum and alpha tocopherol supplied vitamins A, D and E. To the deficient group D was fed the same diet which had been used in Experiment 5 to produce the severe state of deficiency in the young rats. The rats in group D gained weight at a less rapid rate than the rats in the control group

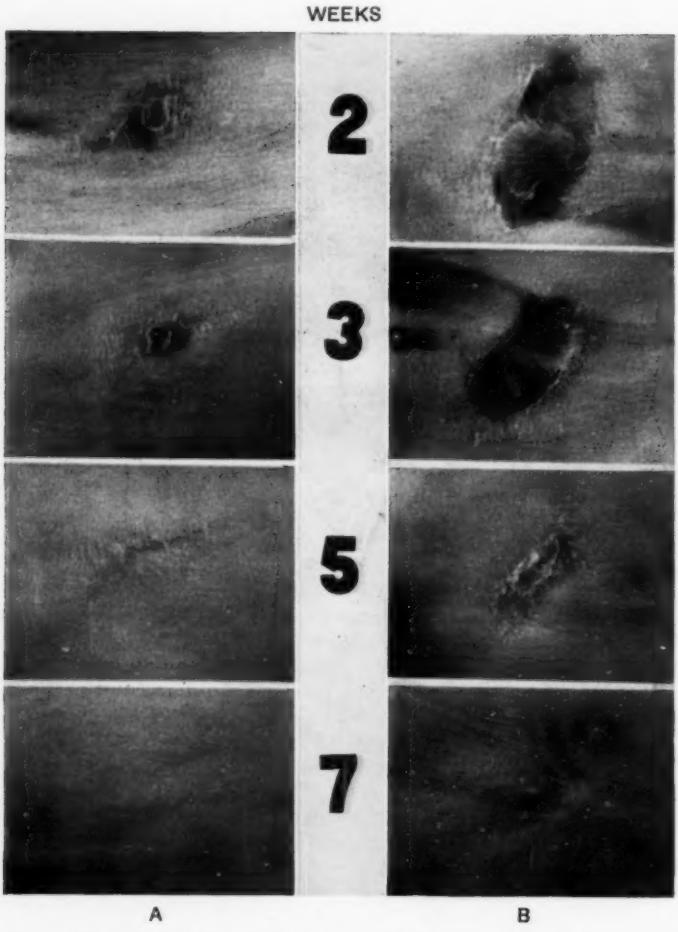


FIG. 1. (Experiment 5.) The serial stages of injury and healing in severe protein deficiency are portrayed at 2, 3, 5, and 7 weeks in the column B. In the control group, column A, left, the extent of injury was less than in the deficient group, column B, right. In the control group the injuries healed in 4 to 5 weeks. In the deficient group healing was not complete at 7 weeks.

C. However, the differences in the weight curves were only slight in contrast to the striking differences in the weight curves in Experiment 5 with young rats. Adult rats may be maintained on this relatively low protein diet (8% casein) for several months whereas the same diet will not support life in recently weaned rats. Between the fourth and fifth weeks after the diet was started, 1.77 mg. of liquid mustard gas was applied to the shaved back of each of 25 rats from group D. Nine rats in group D were not burned in order that they might serve as "deficient controls." All the rats survived. There were no general ill effects after the applications of mustard gas to the deficient rats. The rats in group D continued to gain weight for 2 or 3 weeks after the application of mustard gas. Thereafter the weights were usually stationary. A comparison of the weight curves of the 25 deficient rats that had been burned, and the 9 deficient rats that had not been burned showed no differences. All the deficient rats showed signs of deficiency 8 to 10 weeks after the beginning of the experiment. They were weak; in the majority the fur was unkempt and lacked luster. Animals from each group were sacrificed one week after the application of mustard gas. Microscopic examination of sections from the injured skin and of sections from the spleen, liver and bone marrow disclosed no findings indicating distinctive or characteristic tissue changes in the two groups. Leukocyte counts before and after the application of the gas showed no distinctive differences in the deficient (D) and control (C) groups. Weekly comparisons of injuries in the two groups showed that the extent of injury was approximately equal and that healing occurred with no delay in the deficient group.

Conclusion: The findings in Experiments 5 and 6 indicate that in protein deficiency of an advanced degree the extent of injury due to mustard gas is enhanced and healing time is retarded. Protein deficiency of a mild to moderate degree apparently has no effect on extent of injury and healing time.

FAT DEFICIENCY

Experiment 7: To a group of 30 young rats the following diet was fed: casein 64%, sugar 20%, salts 6%. Into each kilogram of the diet was incorporated: thiamine 20 milligrams, riboflavin 10 milligrams, nicotinic acid 20 milligrams, pantothenic acid 15 milligrams, pyridoxine 10 milligrams, choline 1000 milligrams, inositol 375 milligrams, para-amino benzoic acid 375 milligrams, and cystine 500 milligrams. Vitamin A and vitamin D were supplied by carotene and calciferol and each rat received 10 milligrams of alpha tocopherol per week to furnish vitamin E. During the first 5 months of the experiment there was a gain in weight. After the sixth month the weight was more or less stationary and the rats weighed approximately 120 to 130 grams; there was generalized scaling which was not preceded by or accompanied with erythema or edema; there were loss of elasticity and atrophy of the skin. These are the classical cutaneous signs of fat deficiency in the

rat. Rats of comparable weights were selected for controls, inasmuch as litter mates of the same age would have weighed twice as much. By the rod method small quantities, 180 to 352 micrograms, of liquid mustard gas were applied to the shaved backs of the fat deficient and normal rats. There were no manifestations of general ill effects similar to those observed after the application of larger quantities of liquid mustard gas. Numerous comparative measurements were made, and the results showed that the extent of injury was greater in the deficient rats and that the healing was delayed for 2 to 4 weeks (Figure 2). The scars in the skin of the deficient rats were atrophic and were traversed by telangiectases. Histologic examination revealed no characteristic differences except that the damage was more extensive in the fat deficient rats.

Conclusion: Healing was delayed and extent of injury due to mustard gas was greater in fat deficient than in normal rats.

RIBOFLAVIN DEFICIENCY

Experiment 8: A litter of 10 young rats was divided into 2 groups A, and B. The control group A consisted of 2 animals, and the deficient group B consisted of 8 animals. To the control group A, the following diet was fed: casein 20%, sugar 54%, lard 10%, and salts 6%. Five hundred milligrams of cystine were added to each kilogram of the diet. Oleum percomorphum and alpha tocopherol supplied vitamins A, D, and E. Into each kilogram of the ration the vitamin B complex was incorporated as crystalline supplements in the following amounts: thiamine 20 milligrams, riboflavin 10 milligrams, pyridoxine 10 milligrams, pantothenic acid 15 milligrams, nicotinic acid 30 milligrams, choline 1000 milligrams, inositol 375 milligrams, para-amino benzoic acid 375 milligrams. To the deficient group B was fed a diet similar to that of group A with the exception that the supplement of riboflavin was omitted. In the ensuing month there was a well sustained increase of weight in the control group A. In the deficient group B weight was either stationary or very slightly increased. The rats in group B were humped and weak. Their furs were disheveled and infested with pediculi; these are the classical signs of riboflavin deficiency (6). To the shaved backs of both groups liquid mustard gas was applied. Inasmuch as the deficient rats weighed considerably less than the control rats, the dose was calculated on a weight basis, namely 0.59 milligrams per 25 grams of body weight. The dose administered to the control rats was 1.77 milligrams. The dose administered to the deficient rats was 1.18 milligrams. Three of the rats in the deficient group B died 1 week after liquid mustard gas was applied. The others survived for 6 weeks when they were sacrificed. In spite of the fact that smaller doses of liquid mustard gas were applied to the backs of the deficient group the extent of injury in each case was larger than that in the control group, and healing was delayed 2 to 3 weeks (Figure 3). Erythrocyte and leukocyte counts before and after showed no characteristic differences for the 2 groups. Examination of the spleen and bone marrow disclosed no striking differences in the control and deficient groups.

FIG. 2. (Experiment 7.) In column A, left, the serial stages of injury in a normal rat at 1, 2, 4, 6, and 8 weeks are portrayed. A comparison of the photographs in column B, right, illustrating the injury in a fat deficient rat, shows that the extent of damage is greater in the deficient rat. There was less infiltration in the normal rat's skin than in the deficient rat's skin, although this is not well illustrated in the photographs. Three to 4 weeks after the application of mustard gas to normal rats there was healing with scar formation; in the fat deficient rats crusting and ulceration were extensive at this stage. In 6 to 7 weeks after the injuries, scar formation replaced the eschars in the fat deficient rats, and in 8 weeks healing was complete. Note the delay of healing in the fat deficient rat.

Conclusion: Healing was delayed and the extent of injury was greater in riboflavin deficient rats than in normal rats.

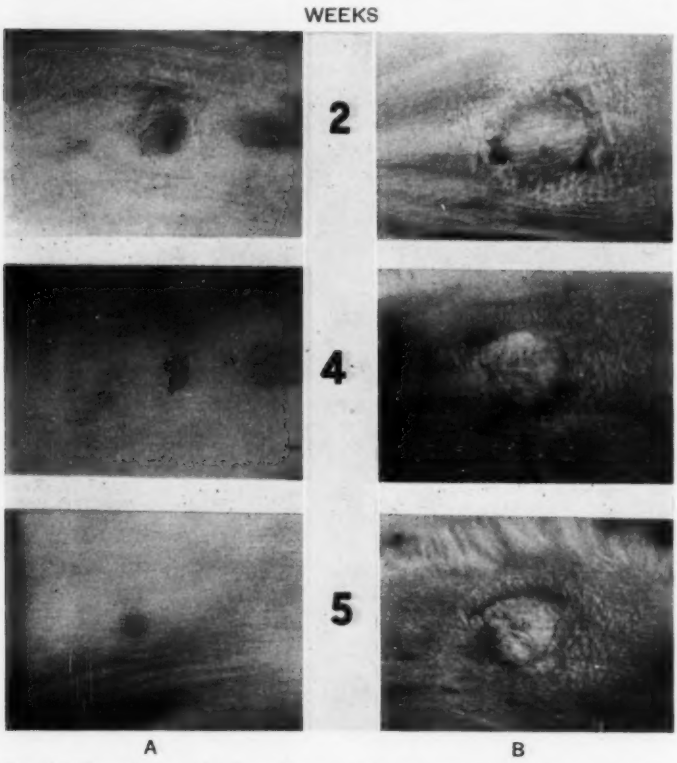


FIG. 3. (Experiment 8.) In column A, left, are portrayed at 2, 4, and 5 weeks the serial stages of injury produced by the application of 1.77 milligrams of liquid mustard gas to a normal rat. In column B, right, are portrayed at 2, 4, and 5 weeks the serial stages of injury produced by the application of 1.18 milligrams of liquid mustard gas to a riboflavin deficient rat. Healing was delayed in the deficient rat and the extent of injury in the deficient rat was much greater than in the normal rats.

PYRIDOXINE DEFICIENCY

Experiment 9: A litter of 10 young rats was divided into group A, consisting of 4 rats, and group B, consisting of 6 rats. To the control group A was fed the fol-

lowing diet: casein 20%, sugar 54%, lard 10%, and salts 6%. Five hundred milligrams of cystine were added to each kilogram of the ration. Oleum percomorphum and alpha tocopherol supplied vitamins A, D, and E. The vitamin B complex was supplied as crystalline supplements and was incorporated into each kilogram of the diet in the following amounts: Thiamine 20 milligrams, riboflavin 10 milligrams, pyridoxine 10 milligrams, nicotinic acid 30 milligrams, pantothenic acid 15 milligrams, choline chloride 1000 milligrams, inositol 375 milligrams, and para-amino benzoic acid 375 milligrams. The diet of the deficient group B was similar except that pyridoxine was omitted. In the ensuing 2 months, there was a well sustained increase of weight in the control group A. In the deficient group B there was a slight increase in weight, and signs of pyridoxine deficiency were manifested by the peripheral symmetric dermatitis which characterizes the disease (7). Inasmuch as the rats in the control group weighed approximately twice as much as those in the deficient group, the dose of mustard gas was calculated on a weight basis. Fifty-nine hundredths milligram of mustard gas per 25 grams of body weight was applied to the shaved back of each rat. The dose administered to the control rats was 2.36 milligrams. The dose administered to the deficient rats was 1.18 milligrams. In the deficient rats, the extent of injury was approximately the same or slightly larger than that of the control animals. However, it should be pointed out that the dose was smaller, so that a smaller lesion should have resulted. Healing was delayed 2 to 3 weeks in the deficient group (Figure 4). There were no systemic ill effects in the deficient group. Erythrocyte and leukocyte determination before and after showed no essential differences in the control and deficient groups. Examinations of the bone marrow and of the spleen showed no striking differences in the control and deficient groups.

Conclusion: Healing was delayed and the extent of cutaneous injury was slightly greater in pyridoxine deficient rats than in normal rats.

PANTOTHENIC ACID DEFICIENCY

Experiment 10: Twenty young rats were divided into 2 groups, A and B. To the control group B the following diet was fed: casein 20%, sugar 54%, lard 10%, and salts 6%. Five hundred milligrams of cystine were added to each kilogram of the diet. Oleum percomorphum and alpha tocopherol supplied vitamins A, D, and E. Into each kilogram of the diet the vitamin B complex was incorporated as crystalline supplements in the following amounts: thiamine 20 milligrams, riboflavin 10 milligrams, pyridoxine 10 milligrams, pantothenic acid 15 milligrams, nicotinic acid 30 milligrams, choline 1000 milligrams, inositol 375 milligrams, para-amino benzoic acid 375 milligrams. To the deficient group B consisting of 16 rats was fed a similar diet with the exception that the supplement of pantothenic acid was omitted. In the ensuing 2 months there was a well-sustained increase in weight in the control group A. In the deficient group B there was only a slight increase in weight. Extensive alopecia, dermatitis and "rusting" occurred in the albinos,

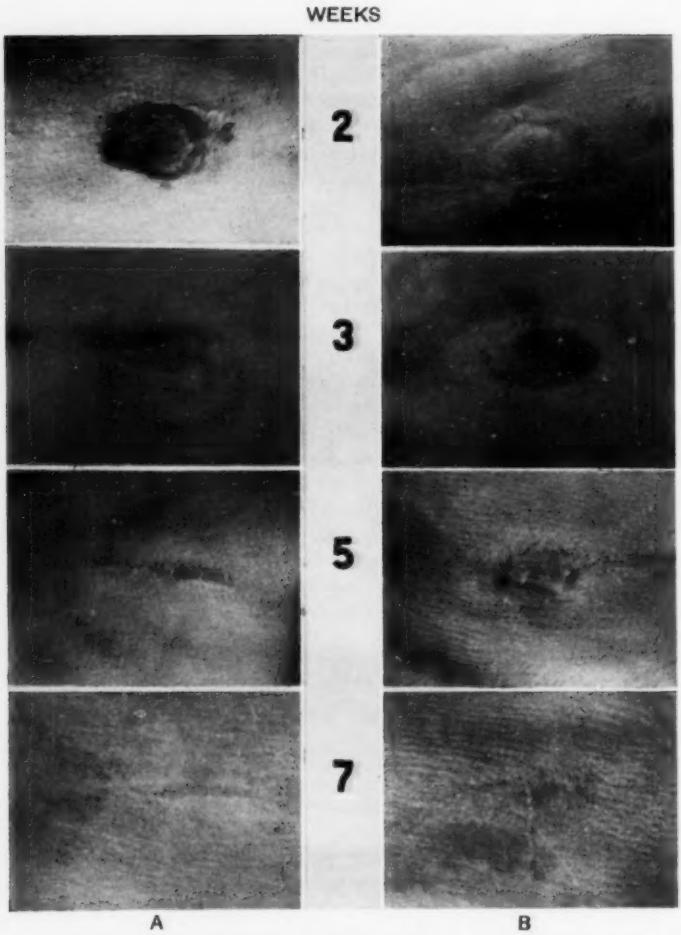


FIG. 4. (Experiment 9.) In column A on the left are portrayed at 2, 3, 5, and 7 weeks stages of injury produced by the application of 2.36 milligrams of mustard gas to a normal rat. In column B on the right are portrayed at 2, 3, 5, and 7 weeks the serial stages of injury produced by the application of 1.18 milligrams of mustard gas to a pyridoxine deficient rat. Healing was delayed for 2 to 3 weeks in the deficient rat and the extent of injury (column B) was slightly greater than that in the normal rat (column A).

and symmetric pattern achromotrichia was observed in the hooded rats; these are the classical signs of pantothenic acid deficiency (8). Because the control rats weighed 2 to 3 times as much as the deficient rats, the doses of mustard gas were calculated on a weight basis. Fifty-nine hundredths milligram per 20 grams of body weight was administered. One and eighteen hundredths milligrams and 1.77 milligrams were applied to the deficient rats, and 2.95 milligrams were applied to the rats of control group A. Weekly measurements showed that injuries which were produced when doses of 1.18 milligrams and 1.77 milligrams of mustard gas were applied to the pantothenic acid deficient rats were slightly larger than the injuries produced by doses of 2.95 milligrams of mustard gas which were applied to the normal rats. Healing time was approximately the same despite the larger dose administered to the control group. Microscopic examination of the bone marrow, spleen and skin of the deficient rats showed no distinguishing features when compared with sections of the control rats. Leukocyte counts before and 3 days after application of mustard gas showed no characteristic differences for the 2 groups.

Experiment 11: A litter of 8 young rats was divided into groups C and D. The control group C consisted of 2 rats, and the deficient group D consisted of 6 rats. To the control group C the diet described in Experiment 10 was fed. To the deficient group D the deficient diet described in Experiment 10 was fed. Two months later doses of 1.77 milligrams of mustard gas were applied to the deficient and control rats in order to evaluate the effect of the same dose in the 2 groups, rather than a dose calculated on a weight basis as in Experiment 10. Weekly measurements showed that the injuries in the deficient rats were greater than those in the control rats, and that healing was delayed for 1 to 2 weeks. Leukocyte counts before and 3 days after showed no characteristic differences in control and deficient groups. Microscopic examination of bone marrow, spleen and skin of deficient rats showed no distinguishing features when compared with sections of the control rats.

Experiment 12: Three young rats that had been depleted of pantothenic acid for 2 months and that manifested the characteristic signs of pantothenic acid deficiency were burned with 1.77 milligrams of mustard gas. Immediately after they recovered from the nembutal anaesthesia administered just before the application of mustard gas, supplements of pantothenic acid were incorporated into the previously deficient diet. During the ensuing weeks signs of the deficiency disappeared and the rats rapidly gained weight. Weekly measurements of the injuries showed that there was an acceleration of healing when compared with the injuries produced by an equal amount administered to the deficient rats in Experiment 11, and the rate of healing was approximately equal to that of the control group, C, in Experiment 11. Blood counts before and after showed no characteristic differences when compared with the leukocyte counts made before and after the application of mustard gas to the untreated animals of Experiment 11.

Conclusion: The findings in Experiments 10, 11, and 12 indicate that pantothenic acid deficiency is conducive to the production of

larger mustard gas injuries. A comparison of the results of these experiments with similar experiments, in which the influence of deficiencies of riboflavin and pyridoxine were studied, shows that the effect of pantothenic acid deficiency on the mustard gas injury was less deleterious than that of riboflavin deficiency or pyridoxine deficiency.

BIOTIN DEFICIENCY

Experiment 13: Biotin deficiency signs were produced in 20 recently weaned rats by feeding a diet composed of cornstarch 177 grams, sucrose 185 grams, dried uncooked commercial eggwhite 300 grams, McCollum salts (No. 51) 58 grams, peanut oil 150 grams. Vitamins A, D, and E were supplied by oleum percomorphum and alpha tocopherol, and thrice weekly each rat received a supplement of thiamin 50 gammas, pyridoxine 50 gammas, pantothenic acid 100 gammas, riboflavine 40 gammas and nicotinic acid 30 gammas. The rats were weak, humped and afflicted with generalized pruritic exfoliative dermatitis. A thick dark brown material consisting of crusts, scales, sebum, broken off hair shafts covered their skin in the fully developed stage of the disease. Doses of 180 to 350 gammas of liquid mustard gas were applied. The thick brown coating appeared to offer protection against the gas as the resulting burns were slight. The brown coating was scraped from the backs of the rats and spread onto the caudal portions of normal rats. Liquid mustard gas was applied to the cephalad portions of the normal rats, as well as to the caudal portion, which were coated with the brown material from the biotin deficient animals. The coating in this circumstance afforded no protection. A mild degree of biotin deficiency was then produced in 16 adult rats. Doses of 1.77 milligrams of mustard were applied. The extent of injury was slightly larger and healing was slightly delayed when compared with injuries in normal litter mates.

Conclusion: Some degree of protection against small doses (180 to 350 gammas) of liquid mustard gas apparently was afforded by the thick coating of scales, crusts, fat, and hair on biotin deficient rats. However, when larger doses (1.77 milligrams) were applied to adult biotin deficient rats in which the thick coating was not present, the extent of injury and healing time were greater than in normal rats. The significance of these findings is not apparent. (See discussion.)

VITAMIN A DEFICIENCY

Experiment 14: After a preliminary unsatisfactory experiment with acute vitamin deficiency in recently weaned rats, it was decided to produce chronic vitamin A deficiency in older rats for this experiment, as the survival period in chronic vitamin A deficiency in adult rats is sufficiently long to make observations over

periods of 2 months or more. Therefore, 8 three months old rats were selected and divided into 2 groups, A and B. To the control group A the following diet was fed: casein 18%, sugar 56%, lard 10%, salts 6%, yeast 10%. Each rat received supplements of oleum percomorphum equivalent to 2800 international units of vitamin A and 400 international units of vitamin D. To the deficient group B was fed the same diet with the exception that the supplement of oleum percomorphum was omitted and vitamin D was supplied as viosterol. During the ensuing 3 months weight gain was approximately equal in the 2 groups. During the fourth month the rats in the deficient group lost weight and ocular and nasal signs of vitamin A deficiency were apparent.

To the shaved back of each rat 1.77 milligrams of mustard gas were applied. Weekly measurements for the next 8 weeks showed no difference in the extent of the injury or healing time of the 2 groups. Microscopic examination of the injured areas of skin showed no distinctive or unusual features in the deficient rats. Leukocyte counts before and three days after the application of mustard gas showed no characteristic differences in the two groups.

Conclusion: The extent of injury due to liquid mustard gas and healing were not affected by vitamin A deficiency in adult rats.

DEFICIENCY OF THE ENTIRE VITAMIN B COMPLEX OTHER THAN THIAMINE

Experiment 15: To 9 rats, each weighing approximately 30 grams and 21 days old, the following diet was fed: casein acid washed 18%, sucrose 66%, butter fat washed 8%, McCollum salts, No. 51 6%, cod liver oil 2%. A supplement of 2 milligrams of thiamine was incorporated into each kilogram of ration. Six control rats were fed the above diet plus 10 per cent Anheuser Busch yeast. In 6 weeks the rats receiving the experimental ration were weak, atrophic and humped. The skin and its appendages were atrophic. Applications of mustard gas were made on the mid parietal regions with stainless steel rods having applicating diameters of 6.25 millimeters. In the 8 control animals, the reaction patterns corresponded to those observed in numerous other normal rats. One day after the application there was noted a localized round or slightly oval area of erythema 10 to 14 millimeters in diameter, in the center of which there was a 6 to 8 millimeter pale area. Two days later there was evidence of exudation in the central portion and peripheral extension of the erythema for a distance of 2 to 4 millimeters. In three to four days there was evidence of exudation in the central portion and peripheral extension of the erythema for a distance of 2 to 4 millimeters. In three to four days there was an increase in the amount of exudation with the formation of a dark brown rather circumscribed crust which was adherent to the skin for 10 to 15 days. The crust was then easily detached or spontaneously came loose. Underlying the crust was a superficial scar of 10 to 15 millimeters in diameter. In 2 or 3 weeks the scars contracted to 5 or 6 millimeters. During all stages of the reaction the extent

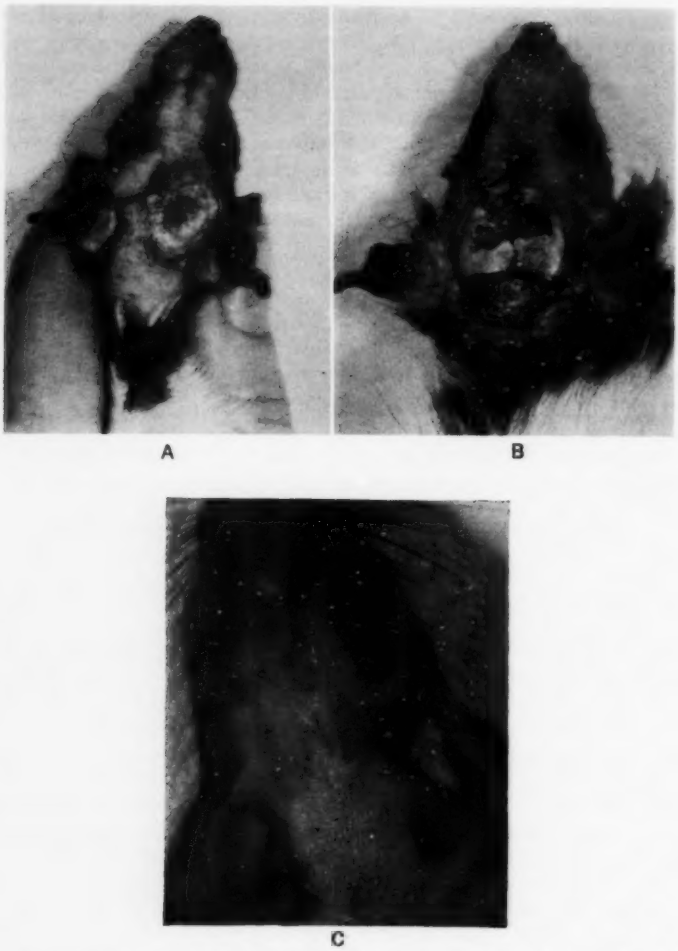
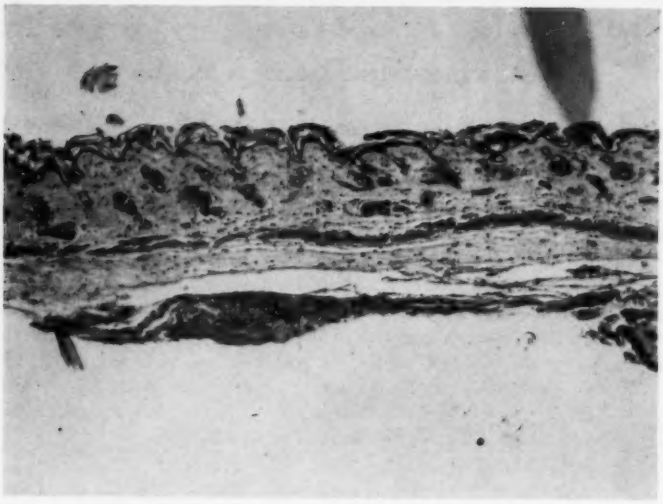


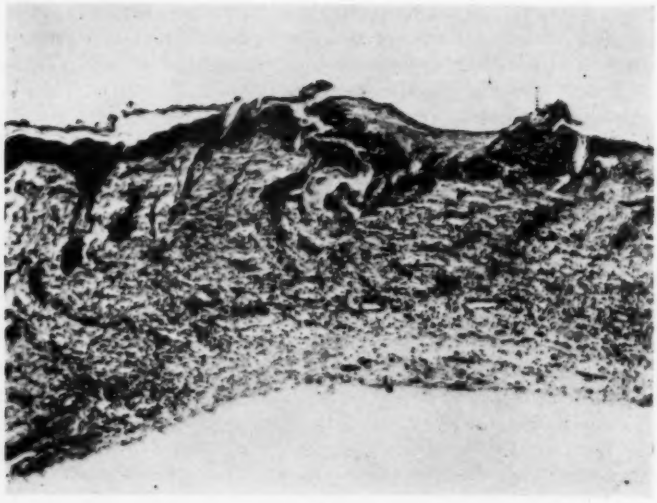
FIG. 5. (Experiment 15.) a. Vitamin B Complex Deficient Rat. Thirty days after the application of mustard gas there was a thick crust and circumjacent erythema. The crust was adherent. The scalp was fixed to the skull. b. When the adherent crust and circumjacent skin were excised injury to the skull was disclosed. c. Normal Rat. Thirty days after the application of mustard gas there was contraction of the scar. It was a slightly atrophic pliable superficial scar.

of the cutaneous alteration was very superficial. The skin was not bound to the deeper structures at any time. The skin was loose and movable in the injured area. It was evident that the injury was confined to the skin (Fig. 5c). By contrast there was a different reaction pattern in the deficient rats. In 24 hours after the application there was diffuse erythema of 16 to 20 or more millimeters. The margins were ill defined and difficult to measure. There was diffuse edema in the erythematous area. One or two days later punctate hemorrhage was observed in the central portion. Irregularly shaped crusts appeared 3 or 4 days after the applications. These increased in thickness and in a week or 10 days the crusted areas were adherent to the underlying tissues. The scalp was bound to the skull and was immobile. In the meantime, the peripheral erythema had subsided. When the crust was forcibly removed, it was evident that the injury had extended down to and had involved the skull (Fig. 5a. and b.).

Microscopic Examination: The epidermis, cutis, and the sebaceous glands were atrophied in the rats that were deficient in the entire vitamin B complex other than thiamine. The skins of the deficient rats were approximately half as thick as the skin of their normal litter mate controls that received 10 per cent yeast as the source of vitamin B. Biopsy sections were taken on the third, fifth, tenth, eighteenth and twenty-fifth days after the application of mustard gas to the heads. The microscopic findings were consistent with the macroscopic signs of injury. The patterns were strikingly different in the deficient rats and the controls. Signs of injury, as well as signs of healing, were delayed in the deficient rats. Three days after the application of mustard gas to the deficient rats it was difficult to detect any signs of injury other than slight edema in the cutis; the atrophic epidermis, hair follicles and sebaceous glands were undisturbed (Fig. 6a). Sections from the normal rats taken 3 days after the application of mustard gas showed extensive alterations in the epidermis, follicles and cutis. There were intraepidermal vesiculation, destruction of epidermal cells and crusting at the site of application. In the cutis there were edema and diffuse cellular infiltration which was often most extensive in the vicinity of the hair follicles and the sebaceous glands. The blood vessels were dilated and hyperemic (Fig. 6b). Five days after the application of mustard gas to the heads of the deficient rats, there was massive edema throughout the cutis, particularly the lower half (Fig. 7a). The blood vessels were dilated and hyperemic. There were no detectable changes in the epidermis. In the control rats at the same stage there was an increase in the destruction of the epidermis and an increase in the crusting (Fig. 7b). Diffuse cellular infiltration persisted in the cutis. The blood vessels were dilated and hyperemic. In 10 days after mustard gas was applied to the heads of the deficient rats the edema subsided considerably and there were disintegrative alterations of the epidermal cells. There was mild diffuse cellular infiltration in the cutis, and the blood vessels were hyperemic (Fig. 8a). Sections taken from the foreheads of control rats on the tenth day showed inter- and intraepidermal vesiculation and acanthosis (Fig. 8b) in some areas and ulcera-

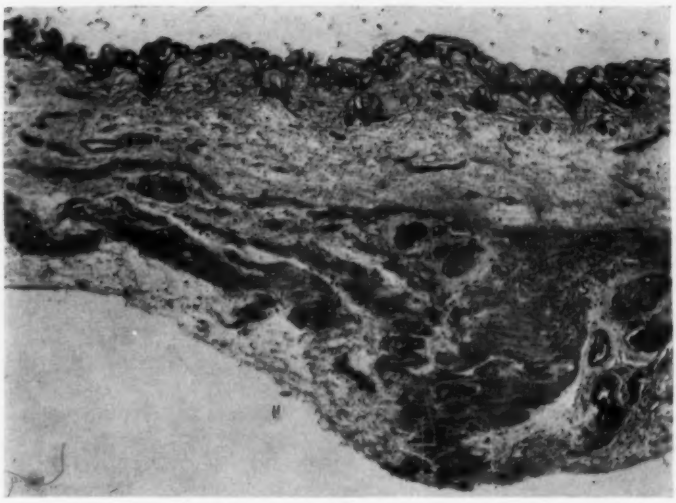


A



B

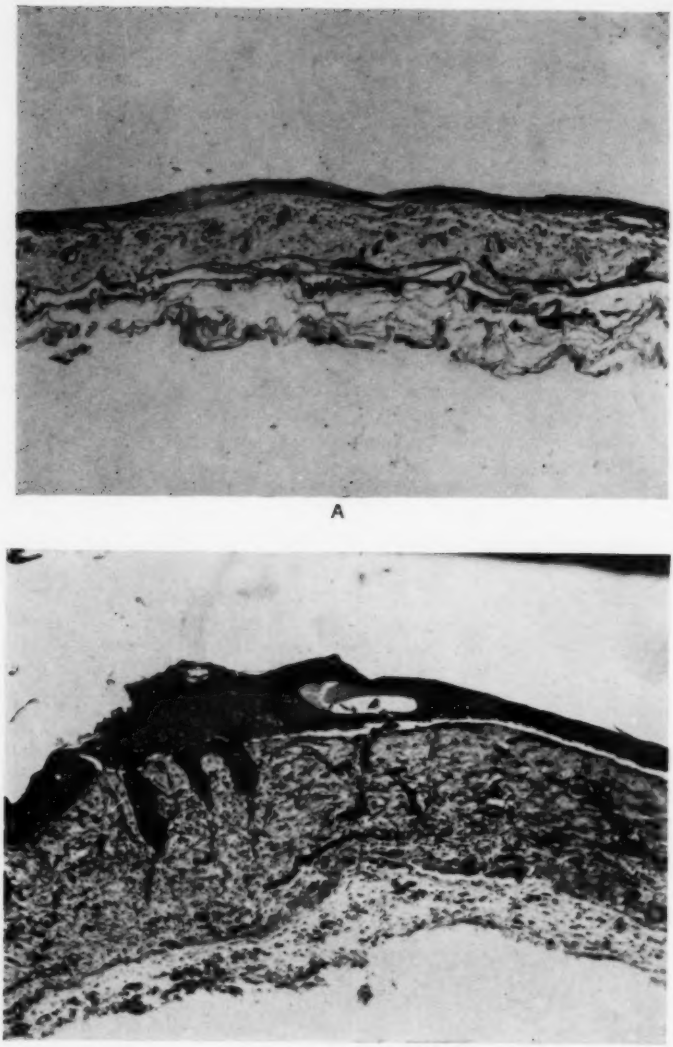
FIG. 6. (Experiment 15.) a. Showing skin of the forehead of vitamin B complex deficient rat 3 days after application of liquid mustard gas. There is atrophy of epidermis, cutis and sebaceous glands. At this stage very slight edema in the cutis is the only sign of cutaneous injury or response to the application of mustard gas. b. Showing the skin of the forehead of the normal control rat 3 days after application of liquid mustard gas. At this stage there is extensive microscopic evidence of cutaneous injury and response to the application of liquid mustard gas. There are intraepidermal vesiculation and crusting at the site of application. In the cutis there are edema and a diffuse cellular infiltration. The vessels are dilated and hyperemic.



A

B

FIG. 7. (Experiment 15.) a. Showing skin of the forehead of vitamin B complex deficient rat 5 days after the application of liquid mustard gas. In the lower half of the cutis there is extensive edema. The blood vessels in the cutis are dilated and hyperemic. The atrophic epidermis at this stage is unaltered. b. Showing skin of the forehead of normal rat 5 days after application of liquid mustard gas. Note the destruction of the epidermis and the thick crust. In the cutis there is a diffuse cellular infiltration which is most extensive in the region of the follicles. The blood vessels are dilated and hyperemic.



A

B

FIG. 8. (Experiment 15.) a. Skin of forehead of rat deficient in the vitamin B complex 10 days after application of liquid mustard gas. The edema has subsided considerably. There are now changes in the epidermis. Hyperemia persists. There is a mild diffuse infiltrate in the cutis. b. Skin of forehead of normal rat 10 days after the application of liquid mustard gas. Note the vesicles in the epidermis. There is less edema in the cutis at this stage than there was 5 days previously (Fig. 7b). There has been a decrease in cellular infiltration.

tion and crusting in other areas. The cellular infiltration and edema in the cutis apparently subsided somewhat during the period from the fifth to the tenth day. Eighteen days after the applications there were extensive crusting and transverse tears in the epidermis of the deficient rats. The atrophic hair follicles immediately below the crusted area were unaffected. The edema in the cutis had partially subsided. In the sections taken on the eighteenth day from control rats there was newly formed connective tissue in the upper cutis. In the region adjacent to the injured area there were no signs of alteration. Twenty-five days after the applications there was complete destruction of the epidermis in the deficient rats (Fig. 9a). There were also extensive changes in the connective tissue. There was diffuse cellular infiltration extending into the subcutaneous region. The muscles also were involved. By contrast, in sections (Fig. 9b) taken on the same day from the normal control animals, the microscopic signs of alteration were confined to a localized portion of the skin and healing had occurred. There was restitution of the epidermis. Scar tissue in the upper half of the cutis replaced the hair follicles and sebaceous glands. The lower half of the cutis showed no changes. The muscle was uninvolved. In the regions circumjacent to the injury there were no signs of alteration.

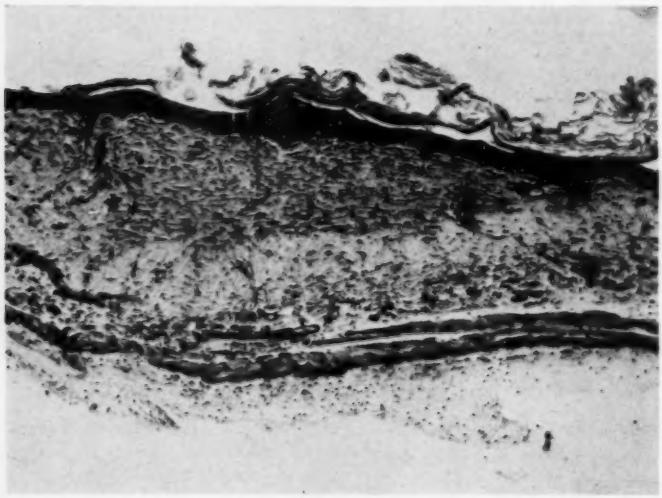
Experiment 16: Experiment 15 was repeated on the mid back of 7 deficient rats and 4 controls in order to determine whether the results would be identical in a different location. The results on the back were similar but not quite as striking as those on the head.

Conclusion: The reaction pattern to mustard gas in rats deficient in the entire B complex other than thiamine differed from the reaction pattern in normal rats. In the deficient rats with atrophic skin the injury extended through the cutis, subcutaneous tissue and the muscle. In normal rats the injury was limited to the skin.

THE INFLUENCE OF CHOLINE AND CYSTINE

Experiment 17: Three litters of young rats were divided into 2 groups, A and B. To the control group A consisting of 6 rats the following diet was fed: casein 20%, sugar 54%, lard 10%, and salts 6%. Five hundred milligrams of cystine was added to each kilogram of the diet. Oleum percomorphum and alpha tocopherol supplied vitamins A, D, and E. Into each kilogram of the ration the vitamin B complex was incorporated as crystalline supplements in the following amounts: thiamine 20 milligrams, riboflavin 10 milligrams, pyridoxine 10 milligrams, pantothenic acid 15 milligrams, nicotinic acid 30 milligrams, choline 1000 milligrams, inositol 375 milligrams, para-amino benzoic acid 375 milligrams. To group B, consisting of 10 rats, was fed a diet and supplements similar to those of group A, except that the supplements of choline and cystine were omitted. Two weeks later

A



B

FIG. 9. (Experiment 15.) a. Showing forehead of vitamin B complex deficient rat 25 days after the application of liquid mustard gas. The atrophic epidermis has been destroyed completely. There are changes in the connective tissue and there is a diffuse cellular infiltration extending deeply into the subcutaneous tissue. The muscle also is involved. b. Showing forehead of normal rat 25 days after the application of mustard gas. In the injured area there is restitution of epidermis. In the upper portion of the cutis there is scar tissue. Note the absence of hair follicles and sebaceous glands in this region. The injury is confined to the skin.

1.77 milligrams of mustard gas was applied to the shaved back of each rat. Comparative weekly measurements for 8 weeks showed that the initial injuries were slightly larger in the rats of group B but healing time was not appreciably affected. Leukocyte counts before and 3 days after the application of mustard gas showed no characteristic differences for the 2 groups.

Experiment 18: Two litters of young rats were divided into 3 groups, C, D, and E. To group C, consisting of 6 rats, was fed the diet described in Experiment 17 with the exception that no cystine was added. To group D, consisting of 8 rats, the above diet and supplements were fed and in addition 1500 milligrams of cystine were incorporated into each kilogram of the ration. To group E, consisting of 4 rats, the above diet and supplements were fed and in addition 3000 milligrams of cystine were incorporated into each kilogram of the ration. One month later 1.77 milligrams of mustard gas was applied to the shaved back of each of the rats. Leukocyte counts before and 3 days after the applications showed no characteristic differences in the 3 groups. Comparative weekly measurements for 8 weeks showed no differences in the extent of injury or the healing time in the three groups.

Conclusion: The findings in Experiments 17 and 18 indicate that omission of choline and cystine from synthetic diet results in slight enhancement of mustard gas injuries; the omission of cystine from the diet had no influence on the extent of injury. Healing time is not affected by omission of choline and cystine together or cystine only. Large doses of cystine added to a synthetic diet failed to accelerate healing.

MAGNESIUM DEFICIENCY

Experiment 19: Magnesium deficiency was produced in 4 litters of young rats by feeding the following diet: casein 200 grams, dextrin 541 grams, butter fat 100 grams, McCollum salts (No. 62) 52 grams. Crystalline supplements of 20 milligrams of thiamine, 20 milligrams of riboflavin, 10 milligrams of pyridoxine, 30 milligrams of nicotinic acid, 15 milligrams of calcium pantothenate, 1 gram of choline hydrochloride, 375 milligrams of inositol, and 375 milligrams of para-amino benzoic acid were incorporated into the diet. Five hundred milligrams of cystine, 18,200 i.u. of vitamin A and 5,460 i.u. of vitamin D were included in the ration. The control diet differed from the deficient diet in that 1 gram of $MgSO_4 \cdot 7H_2O$ was triturated into the salt mixture. The cutaneous signs of magnesium deficiency consist of generalized severe erythema in some animals and multiformed erythematous plaques in others in the early stage, and eczematous dermatitis, edema and thickening of the skin in late stages (9). Liquid mustard gas was applied to erythematous and non erythematous area, to edematous areas and to areas of fissured eczematous dermatitis. There was considerable variation

in the results which were not reproducible. In some animals erythema and/or edema seemed to enhance injury due to mustard gas, but in other animals injuries in erythematous and/or edematous skin were less extensive than those in apparently normal skin areas of magnesium deficient animals as well as in the normal controls. When mustard gas was applied to areas of eczematous dermatitis the injury was not enhanced.

Conclusion: The findings in this experiment were inconclusive. (See discussion.)

THE EFFECT OF FEEDING PARA-AMINO BENZAMIDE

Experiment 20: To one group of adult rats the following diet was fed: casein 20%, sugar 54%, lard 10%, and salts 6%. Five hundred milligrams of cystine were added to each kilogram of the diet. Oleum percomorphum and alpha tocopherol supplied vitamins A, D, and E. Into each kilogram of the diet the vitamin B complex was incorporated as crystalline supplements in the following amounts: thiamine 20 milligrams, riboflavin 10 milligrams, pyridoxine 10 milligrams, pantothenic acid 15 milligrams, nicotinic acid 30 milligrams, choline 1000 milligrams, inositol 375 milligrams, para-amino benzoic acid 375 milligrams. To a similar group of rats was fed the same diet with the exception of para-amino benzamide. Weight curves were approximately equal in the 2 groups. One and seventy-seven hundredths milligrams of mustard gas was applied to the shaved back of each rat. The extent of injury and healing time were equal in each group. The leukocyte counts before and after the burns showed no characteristic differences in the 2 groups.

THE LACK OF INFLUENCE OF MASSIVE DOSES OF CRYSTALLINE VITAMIN B SUPPLEMENTS ON THE EXTENT AND HEALING TIME OF CUTANEOUS INJURY

Experiment 21: To one litter of 7 carefully matched young rats the following diet was fed: casein 20%, lard 10%, sugar 54%, and salts 6%. The ration was supplemented with oleum percomorphum and alpha tocopherol to supply vitamins A, D, and E. Five hundred milligrams of cystine was added to each kilogram of ration. The litter was divided into two groups: A, consisting of 3 rats and B consisting of 4 rats. Into each kilogram of the diet of the control group (A) the vitamin B complex was incorporated as crystalline supplements in the following amounts: thiamine 20 milligrams, riboflavin 10 milligrams, pyridoxine 10 milligrams, pantothenic acid 15 milligrams, nicotinic acid 30 milligrams, choline 1000 milligrams, inositol 375 milligrams, para-amino benzoic acid 375 milligrams. Into the diet of the other group (B) supplements of thiamine, riboflavin, pyridoxine, pantothenic acid, nicotinic acid, inositol, and para-amino benzoic acid were incor-

porated at a level five times as great as that in group A. Two weeks later 1.18 milligrams of mustard gas was applied to the back of each rat. All the rats survived. They were observed for 2 months and comparative measurements were made weekly. The extent of injury and the healing time were identical in each group.

Conclusion: A five fold increase of an adequate supplement of thiamine, riboflavin, pyridoxine, pantothenic acid, nicotinic acid inositol, and para-amino benzoic acid failed to accelerate healing of mustard gas burns.

DISCUSSION

Analysis of the results of the above experiments indicates that atrophy and inanition were factors which enhanced injury and resulted in delayed healing. With the exception of the inconclusive results in the biotin deficient rats, the severity of injury and delay in healing were proportional to the degree of atrophy of the skin. The most severe burns were sustained by the rats that had been deprived of the entire vitamin B complex other than a small supplement of thiamine which was necessary for the maintenance of life (Experiment 15). In this experimental deficiency there is marked atrophy of skin and its appendages and subcutaneous fat is practically absent. Inanition is most pronounced in this combined deficiency. The manner in which liquid mustard gas penetrated through the atrophic skins was in sharp contrast to its action in the skin of the normal control rats where much of the agent apparently was fixed, and the resulting inflammatory reaction was limited to the skin and the subcutaneous tissue. The penetration was of such depth in the scalps of the deficient rats that not only was the musculature involved but there was also necrosis of the skull. Such penetration could be explained simply on a structural basis, that is on the thickness of the skin, but it seems more likely that the atrophic, deficient skin was unable to "fix" the agent because of profound chemical changes secondary to the deficiency. The protective function of epidermal cells and skin appendages is tremendous when the skin maintains its integrity. Subcutaneous fat has been found to possess many biochemical properties and the lipids of fat deposits are constantly subject to a variety of chemical reactions of synthesis, degradation and interconversion

(10). The absence of subcutaneous fat conceivably could account for some of the lack of protection other than by its structural presence. Next in severity to vitamin B complex deficient group were the burns of the riboflavin deficient rats (Experiment 8). The skins of riboflavin deficient rats also are extremely atrophic and subcutaneous fat is sparse. Quantitatively, however, the atrophy and decrease of subcutaneous fat in riboflavin deficient rats is not as severe as in the rats deficient in the entire vitamin B complex (other than thiamine). The other members of the vitamin B complex, namely, pyridoxine, pantothenic acid, nicotinic acid, choline, inositol and para-amino benzoic acid are included in the riboflavin deficient ration and probably are utilized to an extent sufficient to impart to the skin a measure of protective and healing qualities not possessed by the rats deficient in the entire vitamin B complex other than thiamine. Inanition is a prominent symptom of riboflavin deficiency but the general condition and survival of riboflavin deficient rats are better than in the combined deficiency. Next in severity were the burns inflicted on rats with severe protein deficiency (Experiment 5). The skins were atrophic, but were not as thin as the skins of the rats deficient in the entire vitamin B complex other than thiamine and the riboflavin deficient rats. Inanition is not as marked either. Fat deficiency (Experiment 6) should be listed next in the order of deficiencies causing enhancement of injury and delay in healing. The skin in fat deficiency is atrophic but the estimated atrophy is less marked than in the three preceding deficiencies, and the general condition is much better as they survive for many months. Pyridoxine deficiency (Experiment 9) was next in order. In pyridoxine deficiency the skin is only slightly atrophic in the areas tested, that is, on the back, and although underweight their general condition is fairly good until the late stage of the disease. Last in the list of conditions showing a clear cut effect on the extent of injury and healing time was pantothenic acid deficiency, in which atrophy of the skin and loss of subcutaneous fat is also slight, and incidentally in this deficiency there is less evidence of inanition than in the other vitamin B complex deficiencies. Carbohydrate deficiency, vitamin A deficiency, magnesium deficiency and protein deficiency of mild degree exerted no influence on the burns. In these conditions there is only slight atrophy except in the late stages of the deficiencies and inanition is not prominent. In biotin deficiency there is atrophy

of the skin in the fully developed stage of the disease which follows variable stages of hypertrophy of the skin (11). The findings in the biotin deficiency experiment (Experiment 13) are difficult to explain because during the advanced stage of the deficiency, in which a thick coating of scales, crusts, fat and broken hair shafts covered the back (11), some protection was afforded against small doses of liquid mustard. What was responsible for the protection was not determined. When the coating was scraped from the biotin deficient rats and smeared on normal rats it failed to afford protection. In adult rats with mild biotin deficiency the injuries were slightly larger than those in normal controls and healing was slightly delayed. The significance of the findings in biotin deficiency cannot be explained on the basis of the relation of the degree of atrophy to the severity of the injury, as the atrophy in the advanced stage of the deficiency in the young rats was more marked than the degree of atrophy in the mild stage of the disease in adult rats. The findings in the magnesium deficient rats (Experiment 19) were inconclusive, but demonstrated that liquid mustard gas burns were not consistently influenced in either direction by edema, vascular dilation or vesicular dermatitis. These manifestations of inflammation were expected to cause aggravation of injury, but there was great variation in the reactions produced in different groups and the patterns were not reproducible. The lack of aggravation in areas of eczematous dermatitis is difficult to explain as it would be reasonable to predict larger and more slowly healing burns in previously damaged skin. Signs of transient pantothenic acid deficiency were manifest in carbohydrate deficiency when the deficiency of carbohydrate was not compensated for by protein and fat. Signs of pantothenic deficiency have been reported in connection with zinc poisoning (12). The toxicity of mustard gas may have increased the pantothenic acid requirement. However, the results of Experiment 10 indicate that pantothenic acid is less important than riboflavin and pyridoxine in contributing to the protection against and the healing of the vesicant injury.

SUMMARY

1. The application of small measured amounts of liquid mustard gas to the skin of rats results in uniform, reproducible injuries which may be measured precisely and utilized for experimental studies.

2. The pattern of injury in human skin which is manifested by vesiculation differs from that in rat skin in which gross vesiculation never occurs. Hypersensitivity to subsequent exposure of human skin to mustard gas frequently results; hypersensitivity in rats cannot be produced unless previously injured skin is deliberately reinjured.

3. The extent of cutaneous injury is increased and healing is delayed in rats conditioned by deficiencies of the entire vitamin B complex (other than thiamine), riboflavin, pyridoxine, pantothenic acid, protein (severe) and fat. Carbohydrate, vitamin A and mild protein deficiencies exert no influence on the extent of injury or healing time. Cystine, choline and para-amino benzamide had no effect on the healing of mustard gas injuries. Five fold increases of adequate crystalline vitamin B supplements failed to accelerate healing.

4. Inanition and atrophy of the skin appear to influence the extent of injury and the healing time of liquid mustard gas injuries. The severity of injury and delay in healing were proportional to the degree of atrophy of the skin and the grade of inanition. The results in biotin deficient animals were inconclusive and not consistent with the apparent relationship of injury and healing to atrophy and inanition.

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COMPARATIVE EFFICIENCY OF SINGLE AND MULTIPLE DOSAGE REGIMENS OF THE PENICILLINS[1]

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Current practice in the use of penicillin G for the treatment of infections in man demands that a relatively constant blood concentration be maintained. This is generally accomplished by the administration of frequent doses of penicillin G in aqueous solution or by one dose per day of penicillin in oil and beeswax. Both methods are painful for many of the patients, and present other drawbacks such as waste of nursing time, expense, etc. It is pertinent therefore to consider the experimental basis for such modes of administration, in order to appraise how essential is the need for a constant penicillin blood level for the treatment of bacterial infections.

There are three reports available concerning the effectiveness of infrequent dosage of aqueous penicillin upon bacterial infections. Tillett, Cambier and McCormack (1) showed that pneumococcal pneumonia in man could be cured by penicillin, even though the drug was omitted for a 12-hour period during the night. Jawetz (2), using a crude penicillin of unstated composition in the treatment of a hemolytic streptococcus infection in mice, showed that its antibacterial effect lasted eight hours, while penicillin was measurable for only one hour. He suggested a trial of less frequent administration of penicillin in human infections. White, Lee and Alverson (3) found that a single dose of penicillin X would cure 100 per cent of mice with C 203 hemolytic streptococcus peritonitis. White (4), in similar studies with I. M. penicillin X, observed no superiority of multiple dose schedule over single doses.

These reports indicate that in vivo the penicillins exert an antibacterial effect for a longer period than can be explained on the basis of

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the persistence of the penicillin blood concentration. No reports were found which would support the hypothesis that aqueous penicillin G was more efficient as an antibacterial agent either in animals or man, when given at frequent intervals, than when the same total amount of penicillin was given at infrequent intervals. Experiments were therefore undertaken to investigate the comparative efficiency of various dosage regimens of penicillin, using as the test object a virulent hemolytic streptococcus infection in mice, and the pure penicillins G and K as the curative agents. It is to be emphasized that in these experiments, the total amount of penicillin administered to the mice is kept constant, and that only the division of the total dose is varied.

METHODS AND MATERIALS

1. Mice. The mice used for the infections were the CFW strain from Carworth Farms, of uniform stock and age. The weight range for all the mice used was 13–23 grams. However, the weight ranges for the individual experiments were narrower, the figures for the five experiments being 15–20 g., 14–19 g., 14–18 g., 15–23 g. and 13–16 g.

2. Hemolytic Streptococcus Infection. The mice were infected with strain C 203 of a group A, β-hemolytic streptococcus. Stock cultures were grown in trypticase-soy-phosphate rabbit-blood broth, and were kept at 6°C. Virulence was maintained by mouse passage at 2–3 day intervals. Weekly titration of the virulence in mice showed that one organism (or chain), injected intraperitoneally, routinely killed in 24–48 hours. In the experiments mice were infected intraperitoneally with 1.0 ml. of a 10^{-6} dilution of a 5 hour blood broth subculture. Such a dilution contained 1200 to 4500 organisms and killed 78 of 80 control mice in 16 hours $\pm$ 5 hours. The 2 remaining control mice died on the 5th and 7th days respectively. Culture of the heart blood in each instance grew out hemolytic streptococcus. Since it required between 1 and 2 hours to infect 400 mice, in each experiment the first 10 mice and the last 10 mice infected were held as untreated controls.

In the 5th experiment, shown in Table 4, the mice received 12,000 organisms, instead of the average of 2300 for the other 4 experiments.

300–400 Mice were infected for each experiment. In any single experiment 20 mice were used as a unit for each alteration in dosage schedule or amount of dosage. Groups of 4–7 such treatment units

were run for each dosage schedule, and 3–4 dosage schedules were tried in each experiment. In a representative experiment, for example, 6 groups of 20 mice would receive a single dose of penicillin G in amounts ranging from 0.06–2.0mg./k.; 6 groups would receive 3 doses at 8 hour intervals, 6 groups would receive 8 doses at 3 hours intervals, and so on. Reproducibility of results was shown to be of a high order by treatment in each experiment of groups of mice on either a single dose or 3 doses at 8 hour intervals.

TABLE 1

Data on source and purity of penicillin salts used in the experiment

PENICILLIN SALT	LOT NUMBER	SOURCE	IN VITRO POTENCY* UNITS PER MG.	DIFFERENTIAL RATIO $\frac{B.\ subtilis}{S.\ aureus}$	COUNTER-CURRENT INTERPRETATION
Sodium G	J-85-4320	Lilly thru Antibiotic Committee U.S. P.H.S.	—	—	3% K types 2.3% X type Trace F type
Sodium G	V-31	Squibb	1690	0.98	93.5% penicillin G
Sodium K	7/12/46	Pfizer thru Antibiotic Committee U.S.P.H.S.	2075	0.36	95% penicillin K
Ammonium K	Do-320-K	Squibb	2100	0.36	5% other K types

* *S. aureus* cup test.

Only those mice still alive 21 days after completion of treatment were counted as survivors. Cultures were made from heart blood of about half the mice dying later than day 7, and in each instance hemolytic streptococcus grew out.

3. Penicillins. Crystalline salts of penicillin G and penicillin K, in aqueous solution, were used for the treatment of the infected mice. *No penicillin in oil and beeswax was employed.* The purity of the samples was greater than 93 per cent for each salt as shown by the Craig method of counter-current distribution. The data for the 4 salts are shown in Table 1. All the mice in table 3 were treated with penicillin

G-Lilly J-85-4320. Another penicillin G, Squibb V-31, with extensive documentation of purity, showed in vivo no statistically significant difference from the Lilly sample (Table 2).

The penicillin salts were kept in a desiccator at 6°C. Fresh samples were weighed out, on the day preceding the experiment, and were dissolved in 0.9 per cent sterile saline a few hours before use. Injections were made intramuscularly in 0.2 ml. volumes. Treatment was started, in all but one experiment, 4 hours after infection. In the experiment in Table 4, treatment was begun immediately after infection. Dosage was computed in terms of milligrams of penicillin salt per kilogram of mouse.

TABLE 2

Comparative in vivo Activity of Two Samples of Pure Penicillin G

Organism: Hemolytic streptococcus, Group A, Strain C 203.

Infection: Intraperitoneal, 1.0 ml. of 10^{-6} dilution of 5 hour culture, containing 1200 organisms.

Mice: CFW, 15–23 g.

Treatment: Intramuscular sodium penicillin G Lilly J-85-4320 and Squibb V-31 in 0.2 ml. volume. Started 4 hours after infection. Schedule 3 equal doses at 8 hour intervals.

TOTAL AMOUNT OF SODIUM PENICILLIN G ADMINISTERED	PROPORTION OF SURVIVORS		
	Untreated	Treated with Penicillin G Lilly	Treated with Penicillin G Squibb
mg./k.			
1.0	0/20	19/20	17/19
0.5		19/20	16/20
0.25		5/20	5/20
0.12		1/20	1/20

RESULTS

1. Multiple Dose Regimens of Penicillin G. The effect of the various dosage regimens of penicillin G upon the survival rate of infected mice is shown in Table 3. It is important to point out that reading across the table for any mg./k. dosage the total amount of penicillin administered is the same, and only the division of total amount is varied. The comparative efficiency of the different dosage schedules is arbitrarily made on the basis of the calculated Median Survival Dose (SD50). The method of Litchfield and Fertig (5) was employed for the calculation of the SD50 and of the standard error.

The results of the single dose of penicillin G are of such a character

that they cannot be compared with the results of multiple dose schedules, and will be considered separately. The SD50s for the multiple dose regimens, as shown in Table 3, do not differ significantly with the exception of Schedule 5. Hence it seems valid to conclude that, with regard to multiple dose regimens of penicillin G, survival of the mice depends upon the total dose administered. Within certain, but

TABLE 3

Comparative in vivo Efficiency of Various Dosage Regimens of Sodium Penicillin G

Organism: Hemolytic streptococcus, Group A, Strain C 203.

Mice: CFW, 13–23 grams.

Infection: Intraperitoneal, 1.0 ml. of 10^{-5} dilution of 5 hour culture, containing 1200 to 4500 organisms.

Treatment: Intramuscular sodium penicillin G, Lilly J-85-4320, in 0.2 ml. volume, 4 hrs. after infection.

TOTAL AMOUNT OF SODIUM PENICILLIN ADMINISTERED	PROPORTION OF SURVIVORS ON SCHEDULES NUMBER						
	Untreated	I Single Dose	II 4 Doses at 24 Hr. Intervals	III* 3 Doses at 8 Hr. Intervals	IV† 3 Doses at 8 Hr. Intervals	V 8 Doses at 3 Hr. Intervals	VI 8 Doses at 1 Hr. Intervals
mg./k.							
8	0/80	14/20					
4		36/40	18/20				
2		32/39	17/19	17/20	17/20	19/20	
1		27/40	18/20	18/20	38/40	20/20	19/20
0.5		26/40	18/20	18/20	38/40	15/20	20/20
0.25		20/40	5/20‡	8/20	11/40‡	0/19‡	8/20
0.12		8/20		0/20‡	3/40‡	2/20‡	1/20
0.06				0/20‡		2/20‡	1/20
SD 50 (mg./k.)		0.25	0.32	0.29	0.27	0.45	0.24
Standard Error ± mg./k.		0.27	0.03	0.03	0.03	0.04	0.03

* ½ of total dose given at start, remainder divided into ¼–¼.

† 3 equal doses at 8 hr. intervals.

‡ Deaths of > 10 per cent of mice before completion of treatment.

fairly broad limits, the survival rate is not affected by the number of fractions into which this total dose is split, nor by the interval between doses. When the total dose, as shown by Schedule 5, is split into 8 fractions and given at the rate of ⅛ of the total dose every 3 hours, this limit has been passed, and significantly larger amounts of penicillin G are required to save half the mice.

There is one variable which prevents precise comparison of the dosage regimens in the lower dose ranges (0.25 mg./k. or less). With Schedules 2, 4 and 5, as shown by the asterisks in Table 3, significant numbers of mice began to die before the total dosage could be completed. However, the 21-day survival rates for these schedules are

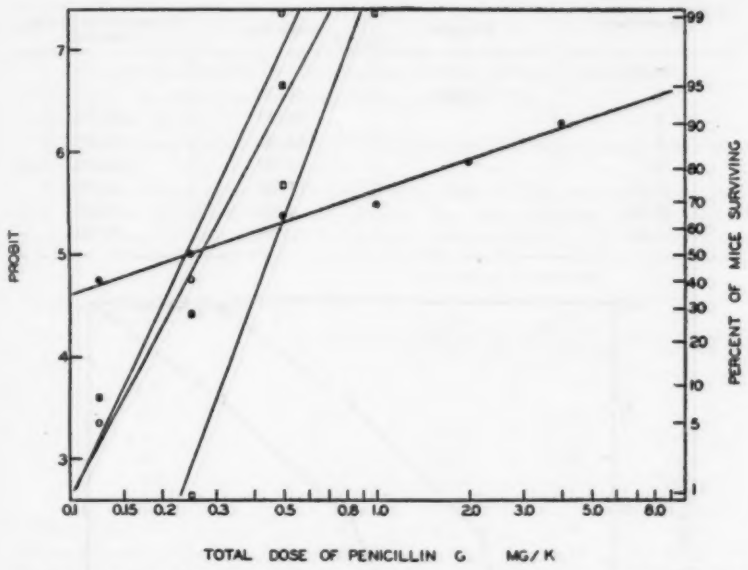

FIG. 1. Effect of intramuscular penicillin G upon survival rate of mice infected with hemolytic streptococcus. Data taken from Table 3.

●——● single doses
○——○ 8 equal doses given at one hour intervals
■——■ 3 equal doses given at 8 hour intervals
□——□ 8 equal doses given at 3 hour intervals

not substantially different from those of schedules 3 and 6, where the mice completed treatment.

2. Single Dose of Penicillin G. The survival rate of mice, treated with various single and multiple dose regimens of penicillin G, is shown in Table 3 and in graphic form in Figure 1. It is noted that a single dose gives a flat type of dosage response curve, i.e., for each increment

TABLE 4

Effect of immediate treatment with sodium penicillin G upon therapeutic effectiveness

Organism: Hemolytic Streptococcus, Group A, Strain C 203.

Mice: CFW, 13–16 g.

Infection: Intraperitoneal, 1.0 ml. of 10^{-5} dilution of 5 hour culture, containing 12000 organisms.

Treatment: Intramuscular, sodium penicillin G Lilly J-85-4320, in 0.2 ml. volume. Treatment started immediately after infection.

TOTAL AMOUNT OF SODIUM PENICILLIN G ADMINISTERED	PROPORTION OF SURVIVORS		
	Untreated	Single Dose	3 Doses Given At 8 Hr. Intervals
mg./k.			
4	0/20	16/20	
2		16/20	20/20
1		12/20	20/20
0.5		7/20	19/20
0.25		11/20	3/20
0.12		4/20	0/20
0.06		0/20	0/20

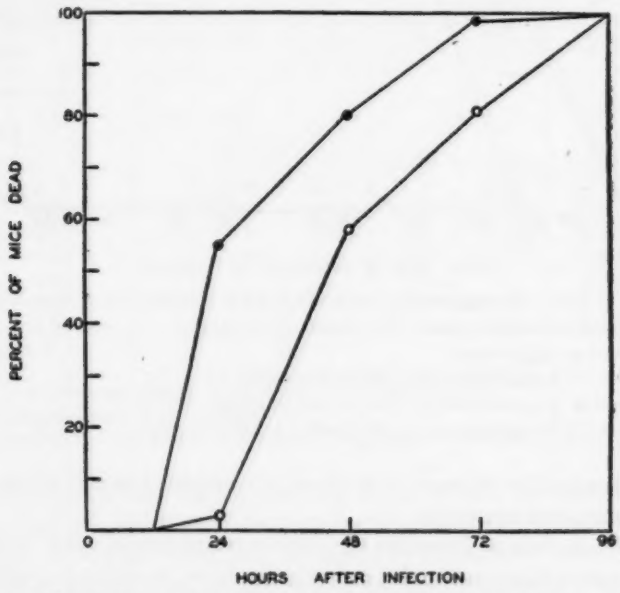

FIG. 2. Survival times of mice with hemolytic streptococcus infection, receiving 0.25 mg./k. of penicillin G in a single dose O——O and in all various multiple dose schedules of Table 3, ●——●. Only mice dying within 96 hours after infection have been charted.

in dosage there is only a small gain in the number of mice surviving. If the SD50 is calculated for this curve, it is the same as the SD50 for multiple doses, but the error for the single dose SD50 is so large that it is not valid to compare the different types of curves. The same type of flat curve results whether the animals are treated immediately or after a 4 hour incubation (Table 4).

TABLE 5

Comparative in vivo Efficiency of Various Dosage Regimens of Penicillin K

Organism: Hemolytic streptococcus, Group A, Strain C 203.

Mice: CFW, 13–23 g.

Infection: Intraperitoneal, 1.0 ml. of 10^{-5} dilution of 5 hr. culture, containing 1200–4500 organisms.

Treatment: Intramuscular sodium penicillin K, Pfizer 7/12/46, and ammonium penicillin K, Squibb DO-320-K, in 0.2 ml. volumes 4 hrs. after infection. Run as part of experiments together with penicillin G, and hence controls are not repeated in this table.

TOTAL AMOUNT OF PENICILLIN K ADMINISTERED	PROPORTION OF SURVIVORS			
	I Single* Dose	II 4 Doses at* 24 Hr. Intervals	III 3 Doses at† 8 Hr. Intervals 1/2–1/4–1/4	IV† 6 Doses at 1 Hr. Intervals
mg./k.				
16	19/20		19/20	
8	20/20	18/20	14/20	16/20
4	19/20	12/20	8/20	7/20
2	11/20	1/20	2/20	0/20
1		0/20	1/20	1/20
SD50 (mg./k.)	1.9	4.0	5.0	5.1
Standard Error ± mg./k.	0.04	0.06	0.12	0.04
$\frac{\text{SD50 K}}{\text{SD50 G}}$	7.6	12.5	17.2	21.0

* Sodium penicillin K, Pfizer, 7/12/46.

† Ammonium penicillin K, Squibb Do-320-K.

Several observations may be made about the effect of a single dose of penicillin G upon hemolytic streptococcus infection of mice.

A. Even with a dose of 8 mg./k., a significant number of mice fail to survive.

B. On the lower end of the curve, the converse is true. That is if 0.12 mg./k. is given as a single dose, 40 per cent of the mice survive; but when the same quantity is divided into 3 to 8 doses, only an occasional mouse survives.

C. In the first 24 hours after a single dose of 0.25 mg./k. of penicillin G, there are practically no deaths (figure 2). With various multiple dosage regimens at 0.25 mg./k., 55 per cent of the mice are dead at the end of 24 hours.

3. The Dosage of Penicillin K. Penicillin K was given to several groups of mice, and the results are shown in Table 5. These mice were treated as part of the same experiments in which penicillin G was used, so that the experimental conditions were identical. The 2 different samples of penicillin K appeared to have the same activity and the results have been pooled.

The SD50 for the multiple dosage regimens is 13 to 21 times that for penicillin G. There is only a slight variation in the SD50 ratio of K to G, with the various modifications in dosage schedules. Not enough data have been collected to decide whether the dosage response curve for a single dose of penicillin K is also flat. The standard error for the SD50 of a single dose of penicillin G is so large that the SD50 ratio of K to G is meaningless.

DISCUSSION

In these experiments with a fulminating streptococcus infection in mice the controlling factor in survival rate is the total dosage of the penicillins. Penicillin G and penicillin K have approximately the same effectiveness as antibacterial agents in mice when given every 8 or 24 hours as when given every hour. Penicillin G blood levels in the mouse, after single intramuscular doses of 4.5 mg./k. and 3.0 mg./k. are maintained for 30 minutes to 1 hour (2, 6). (Valid measurement of penicillin G in the presence of serum cannot be made below a concentration of 0.1 unit per ml. (7)). Comparable doses in man maintain a level above 0.1 unit per ml. for 4 hours (8). On the basis of the data in mice, it seems clear that the antistreptococcal effect of aqueous penicillin G outlasts the measurable blood levels by many hours. In mice, therefore, nothing is gained by administering penicillin G more often than once every 24 hours.

With a given amount of penicillin G, what is the most efficient schedule of administration in hemolytic streptococcal infections in mice? No single answer can be given. A multiple dose schedule would have to be adopted, as a single dose could not be expected to give 100 per cent cures with any reasonable dose. However, a large initial

dose confers an advantage not given by schedules in which the total dose is fractioned into many equal parts. This advantage consists in the fact that a large initial dose protects mice with this fulminating infection for the next 24 hours, whereas with multiple dose schedules, many mice die before they receive enough drug to bring the infection under control.

No final explanation can be assigned for the flat dosage response curve obtained with a single dose of penicillin G. Perhaps it is due to the possibility, as pointed out by Bigger (9) that in any bacterial population a certain percentage of the organisms are in a resting phase. If penicillin G is unable to affect such phases it may be that the flat curve is merely a measure of the number of organisms in the resting phase.

Penicillin K in these experiments has an activity about $\frac{1}{17}$ that of penicillin G. This ratio varies little with alteration in dosage schedule.

These results with penicillin G in mice cannot be directly transferred to man. Nevertheless, it is clear that there is a persistent antibacterial effect of penicillin G, long after its disappearance from the blood. Hence there is little logic in the use of constant blood concentration as the basis for the dosage regimen of penicillin G. Since no one wants to know the minimal effective dose of penicillin in severe infections in man, one should proceed in trial of the above principles in human infections with the use of excess penicillin. Some early results of Tillett et al. (1), in the treatment of pneumococcal pneumonia, indicate that infrequent dosage of penicillin is safe, and probably just as effective as the eight-injections-a-day routine. On the basis of fewer injections for the patient and less work for the nursing staff, one might try, in the treatment of penicillin-susceptible infections, 0.18 g. (300,000 units) of penicillin G intramuscularly every 12 hours. In patients where treatment is begun late and the bacterial population presumably high, it would be important to try the effect of "large" initial doses, say 0.6 gram (1,000,000 units) I. M., immediately and again in 8 or 12 hours, and then smaller doses at 12 hour intervals.

SUMMARY

1. In the treatment of hemolytic streptococcus infections of mice with multiple doses of aqueous penicillin G or of penicillin K, survival

of the mice depends on the total dose administered over a fairly broad range of dosage schedules.

2. Single doses of penicillin G give a flat type of dosage response curve with a large standard error. These results cannot be validly compared with those of multiple dose regimens.

3. Large initial doses of penicillin G are advantageous in so far as they protect mice for a much longer period than multiple small doses.

4. The in vivo activity of pure penicillin K in streptococcal infection in mice is $\frac{1}{15}$ to $\frac{1}{10}$ that of pure penicillin G.

5. Suggestions are made for the applications of these experimental data to the treatment of bacterial infection in man.

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THERAPEUTIC CONFERENCE[1]

The Johns Hopkins School of Medicine and The Johns Hopkins Hospital

Conferences on therapy have been held on alternate Saturdays during 1946–47 and are a joint endeavor of the Departments of Medicine and of Pharmacology and Experimental Therapeutics. The approach to the problems of treatment is based on presenting the fundamental mechanisms of the disease concerned, the physiological action of the pharmacological agents which are used in the treatment of that disease, and our clinical experience in the management of the patient. This rather vertical approach to problems of clinical therapeutics is an effort to place our management of the treatment of the patient on as scientifically sound and rational a basis as possible. Stenotyped reports have been made of these conferences, and a few have been selected for publication in the Bulletin.

THE TREATMENT OF HEART FAILURE

Part I: Digitalis and Its Derivatives

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Dr. A. McGehee Harvey: The conference this morning will be the first of two on various aspects of treatment of heart failure. Today we will take up digitalis and its derivatives and their use in clinical medicine. Dr. Andrus is going to talk briefly first of all about the physiology of the myocardial defect causing congestive heart failure.

Dr. E. Cowles Andrus: Preliminary to discussion of digitalis therapy I propose to rehearse some of the physiological factors which are the basis of what we call congestive heart failure. It is due, at least in its beginning, to the fact that the heart muscle is incompetent to perform effectively the mechanical work imposed upon it. It is a state of diminished mechanical efficiency. The efficiency of any pump or engine is determined, as you recall, by its supply of energy and by its loading. The supply of energy for the myocardium comes to it by

[1] Presented on March 15, 1947 at The Hurd Memorial Hall, The Johns Hopkins Hospital.

way of the coronary arteries; the energy transformations are measurable in terms of oxygen consumption. The energy supply is dependent in some measure upon the integrity of the heart as a pump since this organ provides its own blood flow. The loading is determined by the inflow through the great veins and by the peripheral resistance; the efficiency relative to the loading is influenced by two particular factors, the nervous control of the heart which mediates certain unloading reflexes, and the inherent property of the heart muscle which makes it exquisitely adaptable within certain limits to the load that is thrust upon it.

The response of the heart, efficient and inefficient, to imposed load may be displayed in an isolated denervated preparation, the heart-lung preparation. It is not suggested that the physiology of myocardial insufficiency is altogether as simple as this, but the inherent properties of the heart muscle are the same in the heart-lung preparation and in situ. Moreover there is clinical evidence that "the Law of the Heart" does apply under normal, and under some abnormal, conditions.

In the classic diagram with which you are familiar, the efficiency and the oxygen consumption of the heart-lung preparation are plotted against its work output. With increasing load, oxygen consumption rises; it is almost a linear function of the diastolic length of fiber, or ventricular volume. With increasing load too, the length and the tension of fiber at the beginning of contraction increases. Up to an optimum limit this results in an increased efficiency: increase in work output per unit of energy. However, when the optimum is exceeded, the curve of efficiency flattens out or falls so that beyond the optimum, when additional work is imposed upon the myocardium it is accomplished only at the expense of an extravagant oxygen consumption. This is the essential functional fault in myocardial insufficiency.

Although it is fundamental, this is not the only element responsible for the clinical picture of congestive heart failure. As the methods of clinical physiology have been applied to this condition they have not exonerated the heart muscle but have demonstrated additional abnormalities, such as abnormal salt excretion by the kidney, increased blood volume, etc., which combine to produce the picture and which sometimes dominate it. It is proposed to lift the myocardial fault

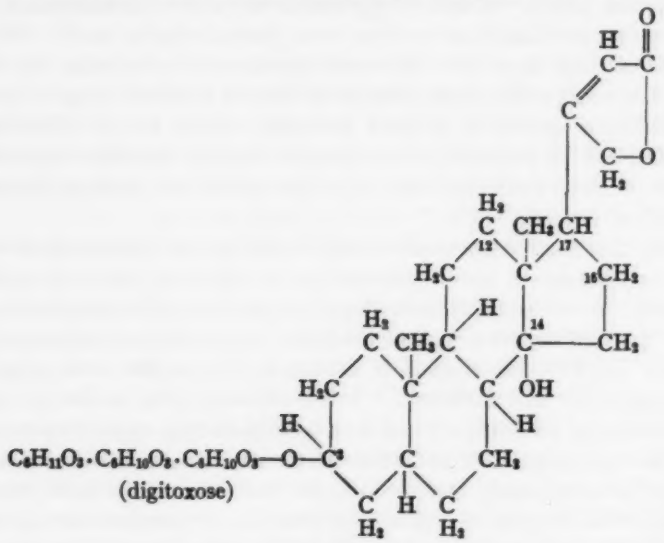
out of its context this morning and to discuss it and its treatment separately.

Dr. Harvey: Dr. Butler is now going to discuss the comparative effectiveness of the various digitalis-like preparations.

Dr. Thomas C. Butler: A digitalis-like action is produced by a number of naturally occurring products, comprising glycosides, alkaloids, and toad venoms. Of these only the glycosides have found any practical use in clinical medicine. Cardioactive glycosides occur in numerous plants. About 30 glycosides have now been isolated in crystalline form and have received some pharmacological study. Only a few of these have been used even experimentally in man. In the last few years several glycosides have become available in pure form in sufficient quantities to allow thorough clinical study. This discussion will be concerned more with the specific differences between some of these glycosides than with the traditional pharmacological effects of digitalis.

The cardioactive glycosides are all closely related chemically, being hydroxylactones of sterol hydrocarbons in which one hydroxyl group is connected with a sugar molecule or a chain of several sugar molecules. The non-carbohydrate part of the molecule is called an aglycone or genin. In 1934 the relation of the cardiac glycosides to the sterols and bile acids was discovered. In the following year or two the advances in the knowledge of the structures were very rapid since much of the knowledge that had been laboriously accumulated for years could be immediately fitted in with the new ideas of the basic structures. The formula of digitoxin is shown as a representative of the type. From the clinical point of view this is one of the most important of the available pure glycosides, although it is now known that digitoxin itself is not one of the native glycosides of *Digitalis purpurea*. A glycoside containing one more molecule of glucose occurs in the leaf and is readily converted to digitoxin by an enzyme also present in the leaf. The aglycones of the other glycosides are quite similar in structure. Several different sugars are found in the glycosides. The glycosides show considerable structural specificity. The genins on intravenous injection have qualitatively the same type of action as the glycosides, but the sugar nevertheless has an important effect. The genins are less active than the glycosides, are not active when given

by mouth, and do not show the delay in onset of action or the difficultly reversible fixation to heart muscle characteristic of the glycosides. The unsaturated lactone ring in the genin is essential for activity. Saturation of the double bond or opening the ring by hydrolysis destroys activity. The hydroxyl in position 14 is also apparently essential for activity. The spatial configuration of the steroid ring system is likewise important. Such apparently minor changes of structure as the introduction of an hydroxyl group in digitoxin in position 16 to



Digitoxin

form gitoxin or in position 12 to form digoxin, although not qualitatively impairing cardiac activity, do alter the quantitative behavior in the body in an important manner that will be discussed later.

The high degree of structural specificity in a rather complex molecule has defeated attempts to synthesize cardiotonic substances. Simple lactones patterned after that part of the molecule lack the characteristic action of digitalis. Progress has been made on the partial synthesis of aglycones from intermediates derived from bile acids and cholesterol, but the synthetic substances so far produced

lack the specific structural features that appear to be essential for activity. Some synthetic derivatives of natural genins have been made in which a carbohydrate other than the naturally occurring one has been attached to the hydroxyl at position 3 or in which that hydroxyl has been esterified with simple carboxylic acids. These compounds are active and are of theoretical if not of practical interest.

The quantitative study of the absorption, excretion, and distribution of the glycosides is handicapped by the lack of chemical methods of sufficient sensitivity. The total dose of digitoxin required to digitalize a man is of the order of 1 mg. A very delicate method indeed would be required to detect such a substance in the concentrations in which it would be present in blood and tissues. Yet without chemical determination and solely by the observation of the physiological effects in appropriate experiments, a surprising amount of information has been gathered about the behavior of these substances in the body.

The various cardiac glycosides, although having qualitatively similar actions on the heart, differ greatly in the following properties, which are of great importance in determining the method of clinical use:

(1) inherent potency
(2) absorption from the intestine
(3) rapidity of onset of action
(4) persistence of action.

The completeness of absorption from the intestine can be judged by comparing the oral with the intravenous dose producing the same effect. It has been found that digitoxin produces almost identical effects by the two routes both in laboratory animals and in man. It must therefore be nearly all absorbed. Ouabain, on the other hand, is almost totally ineffective by mouth. This probably indicates that little is absorbed, although the relative ineffectiveness or oral doses might be in part attributable to the short duration of action of any material that is absorbed. Most of the other glycosides that have been studied in this way appear to be only partially absorbed. The table shows these relationships.

It can be shown on the isolated frog heart or on the mammalian heart-lung preparation that glycosides added to the perfusing liquid do not act immediately. This latent period is different for different glycosides, being much longer for digitoxin than for ouabain. This

time may be the time necessary for the fixation of the drug to the tissue or the delay in the biological response after fixation. If the glycosides are injected intravenously in man, similar differences in the time of onset of action can be observed, the extremes again being represented by digitoxin and ouabain. If ouabain is injected intravenously, changes in the electrocardiogram, in venous pressure, and in the ventricular rate in auricular fibrillation can be detected within a very few minutes and reach their maximum within about half an hour. On the other hand, the effects of intravenous digitoxin come on very much more slowly. They may not be detectable in less than an hour and may reach their maximum only after 12 hours or longer.

TABLE 1*

Showing Quantitative Differences in the Pharmacological Behavior of some of the Pure Glycosides as Observed in Man

GLYCOSIDE	INTRAVENOUS DIGITALIZING DOSE	ORAL DIGITALIZING DOSE	DAILY MAINTENANCE DOSE	TIME BEFORE MAXIMUM EFFECT OF INTRAVENOUS DOSE	DURATION OF ACTION DAYS
	mgm.	mgm.	mgm.	hrs.	
Digitoxin	1.2–2.0	1.2–2.0	0.1 –0.4	2–24	14–21
Digoxin	1.0–2.0	4–8	0.25–0.75	½–2	5–7
Digilanid C	1.0–2.0	5–20	0.5 –2.0	½–2	3–21
Ouabain	0.5–1.0	not effective	0.25–0.5	¼–2	2–3

* This table is based on that of Vander Veer, Med. Clin. North Am., **30**, 1263 (1946), but contains modifications and additions from a number of other sources.

In isolated heart preparations it can be shown that the effects of the glycosides do not disappear rapidly when glycoside-free perfusing liquid is introduced. This phenomenon is usually attributed to the difficultly reversible fixation of the drug to the tissue, but could conceivably be due to the persistence of the physiological effect after the disappearance of the drug. Again, digitoxin and ouabain are the extreme examples, the former washing out the most slowly and the latter the most rapidly. In man the glycosides show the same relation. The effects of a single dose of digitoxin may persist for as long as 3 weeks, while the effects of a dose of ouabain last only a few hours. This persistence of action is the basis of the usual dosage schemes for initiating and maintaining the desired effect.

Thus, the relatively minor differences in chemical structure found in the various cardiac glycosides, although not modifying essentially the qualitative action on the heart, are associated with important quantitative differences in absorption, onset of action, and persistence of action; and it is these differences that determine the field of clinical usefulness of the drugs. For instance, a drug like digitoxin with its complete absorption and persistent action is suited for the routine maintenance of digitalization by oral dosage, while a drug like ouabain is only useful for the rapid initiation of an effect by intravenous injection.

One of the jobs that has always been considered the rightful province of the pharmacologist is the biological assay of digitalis preparations. Almost every common laboratory animal has been used for the assay. Much attention has been given to the standardization of the minutest details of the technical procedures, and the relative merits of the various methods have been argued bitterly. But little attention has been given to the fact that the bioassay of digitalis is a logical impossibility. This is a fact that cannot be evaded by any amount of technical refinement or statistical analysis. Digitalis is a mixture of active principles, and the assay of any such mixture involves either of two assumptions: (1) that the proportions of the several active principles are the same in all samples of the drug; or (2) that the ratio of the therapeutic activity in man to the activity on the test object is the same for each of the active principles. Neither of these assumptions is justified for digitalis. Obviously the amounts of digitoxin and ouabain that are equivalent on intravenous injection in a cat in the official U.S.P. assay cannot be therapeutically equivalent on oral administration to a man.

I do not intend to make the unqualified statement that the bioassay of digitalis has served no useful purpose. Perhaps it has, and perhaps for practical purposes it is good enough. It probably has reduced to some extent the variation of the products on the market. In the usual method of treatment, digitalis is in effect assayed on each individual patient. Large variations would be troublesome, but a very precise knowledge of potency is unnecessary. Particularly in digitalis from the same commercial source, the variations are probably of little practical importance.

Some at least of the logical objections to the assay of digitalis on laboratory animals could be met by performing the assay on man. This is a method that has been advocated by Gold, electrocardiographic changes being used as the criteria of drug action. Whether this procedure would be feasible for large scale use is problematical.

The only logically satisfactory solution to the problem of bioassay of a mixture of active principles is to use one of the pure substances in medicine and so dispense with the necessity of assay altogether. It is to be hoped that this will eventually be the solution of the digitalis problem. Several of the pure glycosides are now commercially available. It is not in my province to pass on the desirability of substituting one or more of these for the crude leaf in medical practice. Some cardiologists think that pure glycosides, particularly digitoxin, do have advantages over the crude mixture. To the pharmacologist it would be gratifying not to have to sacrifice his intellectual integrity by doing a logically impossible bioassay, but this is of course not a sufficient reason. The principal drawback at present to the use of a pure glycoside is economic. The patient buying his drugs on prescription will pay about three times as much for digitoxin as for an equivalent amount of digitalis leaf; but even so his daily maintenance dose of digitoxin will usually only cost him 3 to 10 cents, not a very large item in the budget of many a private patient. The difference assumes more important proportions, however, in the annual budget of a large charity hospital which can buy the crude leaf in large quantities at a very low price. In these institutions, until the price of pure glycosides is considerably reduced, much more convincing arguments would have to be presented for the abandonment of crude digitalis than any theoretical cavilling on the part of a pharmacologist.

Dr. Harvey: Dr. Andrus will now discuss the clinical use of digitalis.

Dr. Andrus: I left the subject of myocardial insufficiency with the heart-lung preparation, and I want to pick it up again there, showing some of the effects of the exhibition of digitalis to such a preparation. In a relatively acute experiment in which myocardial efficiency has fallen to a low level the exhibition of ouabain, for example, increases the work output, the oxygen consumption and the calculated efficiency of the heart. In another preparation in which the diastolic volume of the heart, and therewith its oxygen consumption, has been main-

tained constant, efficiency and work are also increased by ouabain. It has been demonstrated by Starr, among others, that if the volume of the heart is estimated from its frontal area, and the cardiac output is measured, and the work calculated, under normal circumstances there is a direct, apparently optimal, relation between the volume of the heart and the work of the left ventricle. It is also demonstrable that in cases with cardiac disease—some compensated, others uncompensated—the output of work relative to the cardiac volume is diminished.

The clinical effects of digitalis are known to you all. It has a most striking influence upon the rate of ventricular beat in the presence of auricular fibrillation. This effect is certainly not solely the result of vagus stimulation. This chart is taken from a report by Cushny in 1925. It shows the fall of ventricular rate in a patient with auricular fibrillation in two experiments separated by a suitable interval. Strophanthin was given on both occasions, and in the experiment represented by the dotted line it was rapidly followed by an intravenous dose of 2 mg. of atropine. Slowing took place just the same whether the vagus influence was present or not. Another, again one of Cushny's, shows that as the full effect of digitalis is achieved the increase in rate following atropine diminishes. This is restored again after the digitalis effect is permitted to wear off. It can be demonstrated that the acceleration of ventricular rate by exercise is similarly suppressed by full digitalization. This is a point of considerable practical importance in estimating and maintaining the full effect of digitalis. It is best to be certain that the patient with auricular fibrillation receives sufficient digitalis to maintain a ventricular rate within reasonable limits at tolerable activity rather than simply at rest.

I mentioned the fact that cardiac volume and output were optimally related. A second, and probably principally important, effect of digitalis upon the human heart is to increase its systolic contraction and frequently to diminish its size. If 1.8 gm. of digitalis is administered to a patient with fully compensated rheumatic heart disease without evident cardiac enlargement the frontal area of the cardiac shadow in the teleoroentgenograms may be shown to diminish. The effect upon the work and the function of this heart may not be entirely advantageous. The venous pressure may alter very little but circulation

time may rise and cardiac output fall considerably. These are not changes which one would ordinarily associate with improvement of the circulation. In cases of rheumatic heart disease with myocardial insufficiency, there may again be a reduction of the cardiac area and volume after digitalization. But here venous pressure often falls and cardiac output rises. Body weight decreases as diuresis occurs. These changes, it can be shown, bring the relation of cardiac size to cardiac work back toward or into the zone of normal.

In considering the action of digitalis upon the circulation, it is sometimes difficult to distinguish cause and consequence. For example McMichael and Sharpey-Schafer have compared the effects of venesection with those of the intravenous injection of digitoxin. Both procedures bring about a reduction in right auricular pressure and an increase in cardiac work. The same effects can be obtained by trapping blood in the extremities behind tourniquets. The increase in cardiac work with venesection is less than that produced by the digitalis glycosides and more transient. The arterial blood pressure falls following venesection; when digitalis is administered the blood pressure is maintained. These authors interpret their data as showing that digitalis produces its benefit by some effect upon venous inflow and right auricular pressure, reducing the loading of the machine to within tolerable limits. Alternatively the effect might be explained, it seems to me, by an improvement in the competence of the ventricles to put out the blood which is brought to them.

Now nobody knows, as far as I am aware, precisely how these effects of digitalis are brought about. In that sense its administration is still covered with some degree of empiricism, but certain verities have, I think, developed in the course of years. The indication for the use of digitalis, you will all agree, I am sure, is congestive heart failure. Some of the empiricism which characterizes the use of digitalis conceivably results from the fact that patients are not frequently examined or are examined with insufficient care. Digitalis should not be given on a symptomatic basis alone, but only with a full comprehension of the clinical status of the patient. As to choice of preparation, Dr. Butler's remarks are important and enlightening. I personally have had more experience with the powdered leaf than with any other preparation, but digitoxin more recently introduced has certain impressive advantages.

The occasion for the administration of either digitoxin or ouabain by vein is, in my opinion, rare. The emergencies in which lives are saved by this technique are relatively infrequent. Also it is infrequently advantageous to administer a full digitalizing quantity of the drug in a single dose by mouth. In rare instances in which vomiting is frequent, it is advisable to start administering digitalis in the form of cedilanid intramuscularly or digitoxin intravenously in divided doses. Whenever possible it is preferable to rely on the oral route and usually to digitalize in 24 to 48 hours, observing the effect with care.

I think that we would all give digitalis more cautiously if we had to take it ourselves. I remember some very unhappy days which followed my taking 1.4 gram of digitalis in 24 hours. This is an experience that has ever since influenced the rapidity with which I have administered digitalis to a patient. It is not a completely innocuous drug.

As a standard to go by I suggest that it is poor technique to digitalize a patient and to obtain toxic symptoms if it can possibly be avoided. The symptoms of digitalis intoxication are familiar to all of you. The earliest is anorexia. Nausea and vomiting follow soon thereafter and are persistent and annoying. With the typical bigeminal irregularity of the cardiac rhythm, you are again familiar, and, not as a toxic symptom but as a manifestation of the direct action of digitalis, the change in the electrocardiogram.

I prefer to leave the remainder of the session for questions if you care to ask them. One final point I would like to mention which may be brought up again in another connection: the unappreciated danger of the production of overdigitalization if diuresis is produced too rapidly in a fully digitalized patient.

Dr. Harvey: The meeting is now open for questions. I would like to ask Dr. Andrus to give us a resumé of just how he approaches digitalizing a patient—how much to give and with what rapidity to give it.

Dr. Andrus: Considering the use of digitalis as one of several items of treatment let's take a patient with hypertension who has received no digitalis and has acute pulmonary edema. Here I think that one would give a full dose of strophanthin intravenously. It may be that as my experience develops—the experience of others may be greater with it—a relatively large dose of digitoxin would be indicated and again intravenously. Digitoxin does have the important advantage

in contrast to strophanthin that you can translate its effect directly in terms of oral digitoxin if you want to follow with the latter.

Now as to the patient who is orthopneic, breathless and edematous, brought into the hospital having received no digitalis. If the patient is not vomiting I would try to digitalize by mouth in 24 to 48 hours in divided doses. Probably again digitoxin will replace the other products. Personally I would not rush to intravenous digitoxin in that individual if he could take it by mouth. It may be possible that the effects could be more easily achieved that way. I just have an aversion to intravenous medication.

Question: As to figuring out the total dose that is going to be necessary, of course some have complicated rules but we have translated those into more practical rules of thumb. Does that still hold with these new preparations? What is the relative dosage?

Dr. Andrus: I think others who have had more experience with digitoxin may be able to answer that better than I but in the case of the powdered leaf I aim with the ordinary sized individual at 1.2 gram. With digitoxin I suppose it would be 1.2 milligram. However, there is a considerable degree of variation among patients as to the amount required to achieve full therapeutic effect. The physician in charge must guide therapy with digitalis by watching the patient and examining him, stopping short if toxic symptoms supervene or pushing if the effects are not achieved. Finally one has to admit that, sometimes, without the other armamentarium for treating cardiac failure which will be mentioned in more detail in the next session, digitalis is relatively ineffective. You have certainly had that experience. In these cases one can be misguided into producing intoxication because of the lack of therapeutic result. Specifically there is an average but one shouldn't be completely limited by trying to be too quantitative about it.

Dr. Harvey: Does the absorption of digitoxin take place in the presence of passive congestion in the gastro-intestinal tract the same as in the normal individual?

Dr. Andrus: As far as I know. Certainly in some of these patients with auricular fibrillation and congestive failure who have been studied very carefully ventricular rate does go down and to just about the same extent with intravenous and oral doses.

Dr. Harvey: Dr. Baker, have you any comments to make?

Dr. Benjamin M. Baker, Jr.: It is refreshing to hear of a tendency to digress from the practice of precise calculation of digitalis dosage. Patients are individuals and the administration of digitalis to them should be individualized. When digitalis is indicated the largest amount of drug that can be tolerated without signs or symptoms of intoxication should be our goal. One can calculate roughly a safe fraction of the optimal dose and give that rapidly. Having warned the patient of the earliest symptoms of intoxication the remainder can be given in small, frequently repeated amounts.

I would like to encourage the more frequent use of digitalis in patients with enlarged hearts which have not yet failed as one means of postponing the onset of decompensation.

Dr. Harvey: Of course, there were some clinicians who believed that digitalis was not indicated in the absence of auricular fibrillation; it was withheld from patients with heart failure with normal sinus rhythm. I think that idea has gradually disappeared. In certain cases with sinus rhythm it is extremely useful and should be given. The tendency has indeed gone further, and in cases with a severe degree of hypertension even though there is no clinical manifestation of failure except reduction in cardiac reserve, one tends to give an estimated digitalis dose. That is the subject upon which there is a great deal of disagreement. Perhaps Dr. Andrus has something to add.

Dr. Andrus: I am very grateful to you for starting to answer this question. It is one of the most contentious points in the whole subject. I think that there is a trend in treating patients who have cardiac disease but not obvious congestive failure, to explore the possible advantages of digitalizing gradually and maintaining digitalization. This procedure must be fitted to the evident needs of the individual patient. I don't think that one can grant the same amount of justification to the use of digitalis in surgical cases prior to operation simply because the pulse rate is rapid. I am not convinced of the value to anyone of the use of digitalis under those circumstances. On the other hand I believe that if one accepts, as one must, the fact that digitalis can, in the experiment in the heart-lung preparation, reverse and maintain the reversal of a rapidly developing insufficiency, it must be assumed that the drug can maintain cardiac efficiency *in vivo*.

Particularly in cases of hypertension with cardiac enlargement I think there is increasing justification as you say, Dr. Harvey, for examining the benefit of digitalis.

Dr. Charles R. Austrian: I have nothing particular to say except to emphasize what has been said already by Dr. Andrus that, in my experience at least, the frequency of the need for rapid digitalization by vein is generally over-emphasized, for to the majority of patients one can give sufficiently rapid and adequate dose of the drug by mouth. I agree with you that mathematical precision in dosage has been replaced with profit by a "rule of thumb" in many cases. Though for rapid digitalization a total dose of 0.1 gm. per 10 lbs. of body weight suffices when from 25 to 50% of this amount is given initially, I would like to reemphasize the need of individualization in the therapy, to insist that to assume that one can calculate accurately in advance the optimum dosage is misleading, and to point out that maintenance of digitalization is scarcely less important than is the establishment of digitalization.

I think a point raised by Dr. Baker has been bypassed, namely, the so-called tonic medication with digitalis in the sense in which that term was utilized by Dr. Henry Christian. In the prophylactic therapy of the hypertensive heart, it was his idea that there may be sufficient encroachment on the myocardial reserve without the usual objective evidences of myocardial weakness or of failure to constitute an indication for the administration of the drug in doses of 0.05 to 0.1 gm. once or twice daily—an amount insufficient generally to cause unpleasant symptoms. Now whether or not with such minimal dosage, with an amount of the drug insufficient to cause objectively demonstrable digitalis effect in these patients, one accomplishes his purpose, is a moot point and clinical opinion is divided sharply. Different men hold to different ideas. Starting with a skeptical view of the method of Dr. Christian of what might be termed subdigitalizing a hypertensive heart without signs of failure, I have come to believe it has a distinctly beneficial effect.

Dr. Baker: There is important evidence which supports the position Dr. Austrian has taken. Starr has provided convincing proof of digitalis value, in subdigitalizing amounts, by observing serial improvement in the form of ballistocardiograms of patients being slowly digitalized.

Equally significant are the observations of Harrison that attacks of paroxysmal dyspnoea are less frequent in patients receiving digitalis, even in subdigitalizing amounts, than in patients not receiving the drug at all.

I would like to take exception to the position Dr. Andrus took regarding the management of cardiac patients who must be operated upon. No matter how trivial the circulatory abnormality or the operation may be, one can never predict what emergency may arise. Considerable amounts of electrolyte solutions or whole blood may be urgently required postoperatively. It has been amply demonstrated that parenteral fluids may raise the venous pressure of individuals with normal circulations. Therefore to my mind, patients with potential congestive heart failure are in a much better position to face the surgeon and the possibility of urgent intravenous therapy if they are preoperatively digitalized, or at least partially so.

Dr. Harvey: This is not now so urgent as it was before these newer preparations were available. Now we are able to digitalize if necessary very rapidly.

It is one of the most puzzling problems of digitalis therapy that the maintenance dose may vary so tremendously. You see those who take as much as 3 tenths gram of powdered leaf a day and those who get toxic symptoms on 1 tenth of a gram daily. One of the greatest difficulties arises in attempting to determine that level in each patient without having an elusive effect of digitalis on the patients who do require more than the estimated dose of a tenth of a gram per day.

Dr. Andrus: I didn't mean to deny digitalis to a patient going through surgery. I meant particularly that it shouldn't be given for tachycardia alone. All that Dr. Baker has said of course is completely justified. It underscores one other thing: that the use of digitalis requires thorough evaluation of the clinical status of each patient. Wherever possible the venous pressure, circulation time, and the vital capacity should be measured.

Dr. Harvey: Dr. Andrus, I would like to ask a question regarding what I think is another of the difficult situations in which the average student and house officer finds himself. What is the place of digitalis in the treatment of the cardiac arrhythmias, aside from its effect upon the congestive heart failure which may result from such arrhythmias when prolonged?

Dr. Andrus: In the presence of auricular fibrillation you will probably get some control of the irregular rapid ventricular rate by the use of digitalis. This is even more striking in the presence of failure. In the presence of hyperthryroidism and auricular fibrillation you will get relatively little effect from digitalis. In the presence of auricular flutter the effect of digitalis is to increase the rate of the circus movement to an extent to which the auricle cannot respond coordinately and auricular fibrillation supervenes. In the process of digitalization you gain control over the ventricular rate. Frequently, although not invariably, this causes the circulatory status of the patient to improve.

It is not always sufficiently appreciated that digitalis may contribute to the evolution of cardiac arrhythmia which is itself a handicap. Extrasystoles which are so frequent that they produce a considerable pulse deficit are certainly no advantage to circulation. In ventricular tachycardia digitalis is contraindicated; it is likely to make matters worse rather than better. One of the manifestations of severe digitalis poisoning is ventricular fibrillation. I have seen such cases which I am sure resulted from mistaken use of digitalis. In one the patient took an overdose by error.

Dr. Harvey: How about the situation in patients with myocarditis as far as digitalis is concerned?

Dr. Andrus: It would be my impression that if you are satisfied with that diagnosis digitalis would not be indicated.

Dr. Harvey: Of course that brings up the problem of the place of digitalis in the treatment of severe infections including pneumonia. Is there any increased susceptibility to digitalis in these people?

Dr. Andrus: I can't prove it but it is my impression that there is. I think oxygen would do the heart more good than digitalis in those circumstances.

Dr. Baker: The use of digitalis in pneumonia remains a controversial subject. Twenty years ago in this hospital it was given to almost every patient with lobar pneumonia. Gradually we have developed a clearer understanding of the common circulatory disturbance in this important disease; peripheral circulatory failure is relatively common and congestive heart failure is rare. Digitalis is contraindicated in peripheral circulatory failure and hence is rarely indicated in patients with lobar pneumonia unless they have pre-existing cardiac disease.

Dr. Andrus has pointed out that digitalis produces certain cardiac arrhythmias. So does anoxemia and the digitalized, cyanotic patient with lobar pneumonia or bronchial asthma is a good candidate for cardiac arrhythmias. Before giving digitalis to patients with either of these conditions I advocate consideration, among other things, of frequent determinations of the venous pressure.

Dr. Austrian: Opinion concerning the indications for and the efficacy of digitalis in acute lobar pneumonia has varied greatly through the last few decades, influenced especially when the differentiation was established between the circulatory failure of peripheral as distinguished from that of cardiac origin. Some forty years ago in this clinic a man's tenure as a house officer was jeopardized if he gave digitalis to a patient with lobar pneumonia as a routine procedure, that is in the absence of some identifiable evidence of myocardial embarrassment. Tachycardia alone—without arrhythmia newly developed, falling blood pressure, etc.—was not regarded sufficient indication for exhibition of the drug. That practice prevailed until some short while after work from the Hospital of the Rockefeller Institute seemed to establish the desirability of administering digitalis to every patient with lobar pneumonia, when it was exchanged for such routine administration. Then, once again after it had been established that the drug did not influence the toxic tachycardia and that it even lessened the minute volume output of the undamaged myocardium, routine administration was frowned upon and individualization of the medication was established as the proper method of treatment. From this vacillation there developed the need first to establish the circulatory efficiency; secondly, if there was circulatory failure whether it was of peripheral vascular or of cardiac origin; and thirdly, only if evidence of myocardial defect were developing or apparent was digitalization to be induced.

Dr. Harvey: Does anyone else have a question?

Dr. Nathan B. Herman: Will you comment on the use of digitalis in bronchial asthma in the absence of true cardiac damage.

Dr. Andrus: Speaking in the abstract, I think they ought to have all the support they can get. That is one of the instances in which it might be an advantage rather than a disadvantage to use digitalis.

Question: What about the use of digitalis in the failing heart undigitalized but with many ventricular extrasystoles?

Dr. Andrus: You not infrequently see that the extrasystoles diminish as digitalis takes effect under those circumstances.

Dr. Baker: I would like to put in a plea for the use of quinidine under those circumstances.

Question: Is there anything against the use of quinidine with digitalis with heart failure in the presence of extrasystoles?

Dr. Andrus: Not that I know of. There is one situation in which we used to feel that the two shouldn't be given together because of their antagonistic action on the auricular muscle; when we were trying to revert auricular fibrillation.

Dr. Harvey: I am one of those people who still considers quinidine a dangerous drug.

Dr. Andrus: So am I.

Dr. Baker: I would like to add further emphasis to the need for caution in the use of quinidine. Although I am sure there is danger in the use of quinidine I am equally afraid of ventricular extrasystoles in a patient who has recently had myocardial infarction. I certainly advocate the administration of quinidine to patients in this fix and the withholding of digitalis as long as possible because of the increased ventricular irritability it may induce.

Dr. Harvey: To attempt to revert auricular fibrillation of long standing with quinidine is, I think, bad procedure for several reasons. In almost no instance does the rhythm revert to normal and remain so. The patient may be very little better off as far as the cardiac function is concerned.

Summary: In the course of the conference, pharmacologists and clinicians have discussed one of our most useful drugs. The discussion has brought out the lack of knowledge of the mechanism of the action of digitalis despite the fact that it has been extensively employed for more than 150 years in the treatment of "cardiac dropsy". Digitalis has been prepared for use in increasingly pure form, but the dose which produces beneficial effects and that which causes toxic symptoms are still inconveniently close together. Repeated attempts have been

made to design a convenient routine for digitalis therapy. As matters stand at present, to obtain its full benefit the administration of digitalis must be guided by careful preliminary evaluation and periodic re-evaluation of the patient's condition.

The digitalis bodies provide a challenging field for collaborative study by pharmacologists and clinicians. It is not too much to hope that by such future collaboration the mechanism of the action of these important compounds may be elucidated and their usefulness correspondingly increased.

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THERAPEUTIC CONFERENCE
THE TREATMENT OF HEART FAILURE

Part II. The Use of Diuretics

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Dr. Harvey: As a continuation of the last conference on the treatment of congestive heart failure, in which digitalis was discussed, we are now turning to the question of diuretic therapy. Dr. Newman will open with a discussion of the physiology and pharmacology of diuretic drugs.

Dr. Elliott V. Newman: The first thing necessary in a discussion of diuretic drugs in cardiac failure is to define what we mean by the word diuresis. As clinicians concerned with diuresis we are primarily interested in removal of fluid from the body, producing negative fluid balance. The abnormal collection of body fluid in cardiac failure can be considered for practical purposes as extracellular fluid which consists of water, sodium, and chloride in constant proportions. So when we speak of a diuretic drug or of diuresis, we imply a substance or process which removes water and sodium chloride and leaves the composition of the remaining fluid in a relatively normal state. The drugs that we use may primarily affect water and, secondarily, salt excretion, or they may primarily affect the excretion of salt and, secondarily, water. Since the composition of body fluid is very closely guarded by the kidney, when one substance is deranged, the kidney usually rapidly readjusts proportions by excreting the related substances.

I plan to discuss diuretic drugs from the physiological point of view under three headings—first, the "acid-producing" salts; second, the xanthines; and, third, the mercurials. Finally I might comment on some of the factors involved in the use of a low salt diet with a large water intake to promote the loss of edema fluid.

I. The "Acid-Producing" Salts

I am going to discuss ammonium chloride as an example because it is commonly used. The history of the acid salts is interesting. They were discovered in this century by German workers who gave calcium

chloride to children to reduce albuminuria, and noticed that they lost weight. Later, about 1920, Haldane used ammonium chloride to improve high altitude tolerance; and in his charts one notices that a marked diuretic effect followed its administration. It was suggested later that calcium chloride and ammonium chloride might be used to remove body fluid. Then the well-known work of Gamble appeared on the effects of calcium chloride and ammonium chloride administration on electrolyte excretion.

Ammonium chloride is usually given by mouth and in enteric coated tablets to prevent gastric irritation. Absorption is rapid and nearly complete in three to six hours, so it is usually given four or five times a day.[1] After absorption from the intestine the NH_4 ion becomes intimately involved in nitrogen metabolism, disappearing from the blood rapidly. Thus the chloride ion remains and in effect the patient has received hydrochloric acid. The excess chloride ion is excreted by the kidney and thus requires the excretion of base. When the base lost is sodium a proportionate amount of body water is also excreted.

It is instructive to make a brief calculation of the effect of ammonium chloride assuming it were completely efficient; that is, if one molecule of ammonium chloride removed one of sodium. One milliequivalent of ammonium chloride equals .053 grams. Assuming there are 20 liters of extracellular fluid in the average body and 140 milliequivalents of sodium per liter, we have 2800 milliequivalents of sodium in the extracellular fluid of an average person. The question is: How much ammonium chloride would it take to remove all the sodium in a patient's extracellular fluid? The quantity amounts to (.053) (2800) or 148 grams of ammonium chloride: or roughly 10 grams of ammonium chloride a day for two weeks.

Fortunately, the kidney has some defense mechanisms which limit the effectiveness of ammonium chloride and these have an important bearing upon the way in which ammonium chloride should be administered. The base-saving mechanisms of the kidney may largely vitiate the desired action of ammonium chloride. First, the kidney can excrete some of the acid as such, but this mechanism is of limited capacity. More important is ammonium production by the kidney

[1] Often the ineffectiveness of ammonium chloride administration is explained by the appearance of unchanged enteric coated tablets in patient's stools.

which reduces loss of sodium, because the excess chloride is excreted as ammonium chloride instead of sodium chloride. The dynamics of the ammonia-producing mechanism is important. If one gives 8 to 10 grams of ammonium chloride a day to a normal person, on the first day the sodium loss is marked. The second day the loss is not as large and by the third sodium excretion may be nearly normal. If we plot the amount of ammonia produced by the kidney each day, we find that the increase in ammonia production is a sluggish mechanism. Ammonia production rises slowly on the first day and reaches a peak, usually by the third day. And as ammonia production increases there is less sodium loss proportionately. The importance of this is that with normal renal function, initial large doses are more effective than small prolonged doses, since the drug is more efficient in the first few days before ammonia production is fully stimulated.

The drug is more potent and may be much more dangerous with damaged kidneys which do not have a normal ammonia-forming mechanism, because sodium loss can be tremendous. Serious uncompensated derangements of body fluid composition may occur. The kidney may not excrete the chloride. There may be overall depression of function with a low filtration rate and chloride may be retained, producing what may be called a "chloride acidosis". There are changes in the intracellular electrolytes with ammonium chloride administration with a marked loss of phosphorus and potassium, which presumably come from the cells. Also with dehydration and acidosis not only extracellular fluid but intracellular fluid is lost. Thus prolonged administration of "acid producing" diuretic salts is dangerous particularly in a patient with damaged kidneys.

II. The Xanthine Diuretics, Caffeine, Theobromine, and Theophyllin

The xanthines were discovered and first used as diuretics in 1890 by Von Schroeder, and it is worthwhile reviewing some of the things that were discovered by the original investigators. I will omit discussion of caffeine, since it is not usually used as a diuretic. Experimental work on dogs and rabbits with theobromine and theophyllin showed that they were active diuretics, but that their potency varied in different animals. I think it is well to keep in mind in evaluating studies on diuretics, that the cat, rat, rabbit, and human may vary markedly

in their response and that one cannot freely carry experimental conclusions derived from one species to another. Koritschoner and Gram in 1890 pointed out that theobromine showed no toxicity in patients in doses up to 4 to 5 grams a day, and that the theobromine sodium salicylate salt was more soluble than the free compound. They found that theophyllin was a more potent diuretic than theobromine but produced central nervous system, cardiac, and gastro-intestinal disturbances, such as excitement, cardiac irregularities, diarrhea, and nausea and vomiting. Grunwald in 1908 and Butler and Kerpel Fronius in 1935 studied the effect of theobromine on electrolyte balance in rabbits. They found that there was marked loss of sodium chloride and potassium in these animals; the serum became concentrated; there was weight loss; serum non-protein nitrogen rose; and the animals died with a progressive ascending paralysis of the limbs.

There have been two general theories of the mechanism of action of the xanthine diuretics. First that the diuresis is due to an increased renal blood flow; second, that the xanthines cause a decrease in absorption of electrolytes and water in the renal tubule. Without going extensively into the evidence, I think one can be fairly certain that theobromine and theophyllin act on the renal tubule by decreasing reabsorption of sodium chloride. I mention sodium chloride loss first, because the amount of water lost with the xanthines in animals and in patients both with edema and without edema is variable.

One can block the water loss due to xanthines with pitressin but sodium chloride loss in the urine will be as great as with the xanthine alone. The most consistent effect of the xanthines is an increased salt excretion.

Experiments in our laboratory with theophyllin show that the renal blood flow and filtration rate in the dog do not increase. However, an increase in urine flow did occur but it was not the most dramatic effect. The striking effect was the marked increase in the output of sodium chloride due to decreased tubular reabsorption (Fig. 1).

I have selected for comment three clinical evaluations of the xanthines; one by the first group to use xanthines in 1890, the second by Christian in 1916, and the third by Marvin in 1926. Koritschoner and Gram in 1890 studied a series of 38 cases of dropsy, 10 with chronic valvular heart disease, 6 with arteriosclerotic heart disease, 4 with

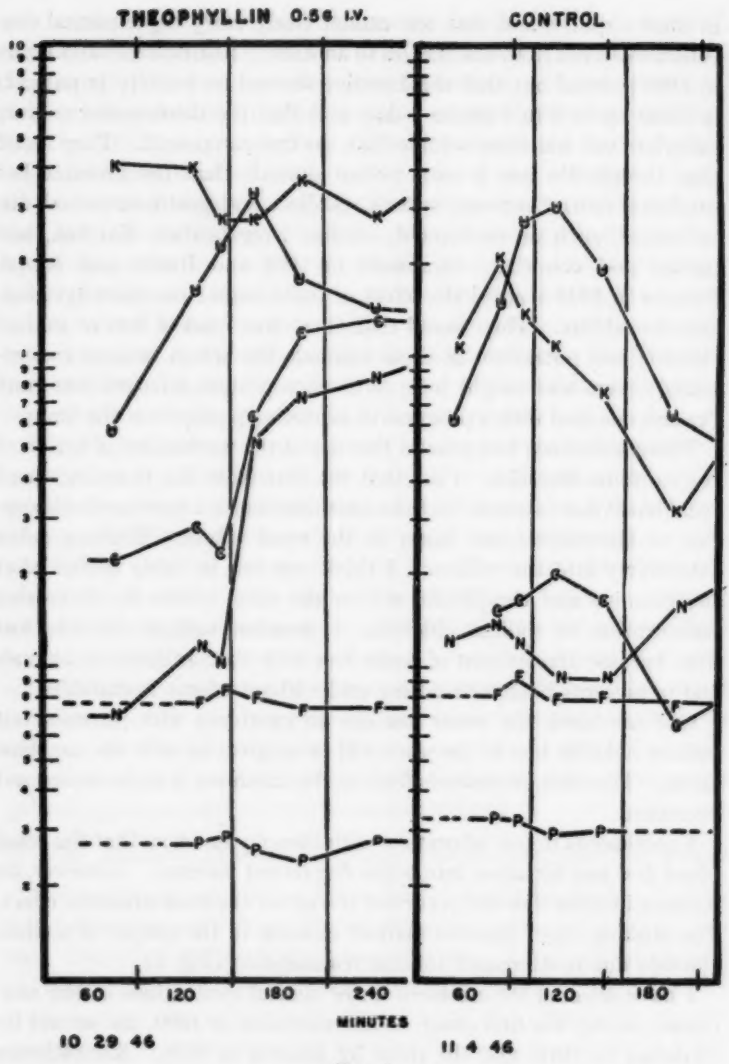

FIG. 1.

P—Renal plasma flow (para-amino hippuric acid clearance)
F—Glomerular filtration rate (exogenous creatinine clearance)

degeneration of the myocardium, 3 with cirrhosis of the liver, and 15 with nephritis. They used 4 grams of theobromine a day and concluded that 23 of the 38 patients were markedly benefited, and that the action in the cardiac cases with dropsy was much more effective than in the nephritics. About 1916 Christian conducted a series of experimental and clinical studies on the xanthines. He pointed out that patients who had no edema lost chloride with theophyllin even though there was no diuresis. In five patients with chronic nephritis, 4 of whom had edema, he obtained slight diuresis with 0.3 to 1.0 grams of theophyllin per day, but in all patients there was a marked increase in chloride excretion. He then studied six patients with chronic cardiorenal disease, using theophyllin, 0.3 to 1.5 grams per day, and

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K—Potassium clearance
U—Urine flow
N—Sodium clearance
C—Chloride clearance

The control experiment on the right was made omitting theophyllin to determine any effect of oral creatinine or para-amino hippuric acid infusion. The hour marked by the vertical lines is the comparable time during which theophyllin was infused in the experiment on the left.

The ordinate is a triple cycle logarithmic scale in order that percentage changes in the values are easily compared.

An increase in urine flow occurred in the control experiment probably due to the infusion of para-amino hippuric acid solution, but there were insignificant changes in sodium and chloride clearance during the first two hours.

On the left the specific effect of theophyllin upon sodium and chloride reabsorption in the renal tubule is demonstrated.

During the hour marked by vertical lines 0.5 gram of theophyllin was added to the para-amino hippuric acid infusion, so that the conditions of the experiment were otherwise exactly as on the control day.

During theophyllin infusion, filtration rate (F) and renal plasma flow (P) did not change significantly.

There was a striking increase (nearly ten fold) in the sodium and chloride clearances occurring immediately. Urine flow increased but not significantly more than in the control experiment, and potassium clearance remained approximately the same.

Such a marked increase in sodium and chloride clearance without change in filtration rate is due to decreased reabsorption of sodium and chloride by the renal tubular cells.

Fasting unanesthetized dog on a constant diet. Urine collections made by inlying catheter.

produced a marked diuresis in four but no diuresis in two who were edema free at the time of administration. There was a marked increase in sodium chloride excretion in those having a diuresis. He concluded at that time that the xanthines were not beneficial in nephritis, but were efficacious in cardiac edema.

In 1926 Marvin studied the effect of diuretics in 77 patients with congestive cardiac failure. He eliminated 36 of the patients who became edema-free on digitalis alone. Of the 41 patients remaining, 23 were benefited with theobromine or theophyllin. He found that theophyllin was more effective than theobromine and that theobromine was sometimes more effective than theobromine sodium salicylate. The patients had untoward reactions, such as nausea and vomiting and some mental excitability with theophyllin, but none with theobromine.

III. The Mercurial Diuretics

The organic mercurials were first used in 1917 by Zeiler and rapidly were heralded as the most powerful diuretics. Mercurial diuretics are usually given by vein or intramuscularly and recently have been given by mouth and rectally. I think most of us use them intravenously or intramuscularly. There has been some recent interesting work on the absorption of the mercurials from muscle. As you know, it is customary to put theophyllin in mercurial preparations. It has been shown by DeGraff that theophyllin mixed with mercurial preparations markedly increases the rate of absorption from the muscle site. Occasionally the effectiveness of mercurials is poor when given intramuscularly, probably because of slow absorption from the muscle in an edematous patient with circulatory failure. Administration by rectal suppository is a convenient and sometimes satisfactory method; but not as uniformly effective as administration intravenously or intramuscularly.

There have been three main theories regarding the mechanism of action of mercurial diuretics. One theory that we can dismiss, I think, is that the mercurials primarily cause mobilization of salt and water from the tissues. The other theories place the site of action in the kidney. Renal blood flow may be increased or there may be decreased reabsorption of salt and water from the renal tubule. The work of Govaerts placed the site of action in the kidney. He showed that if

mercurials were injected into one kidney which was then transplanted to the neck of another animal, the diuresis continued. With the clearance techniques it is found that the mercurials do not primarily affect renal blood flow or filtration rate, but cause a specific decrease of tubular reabsorption of salt and water.

The experimental work of Blumgart and others demonstrates the overall effect of intravenous mercurial injections in normal and edematous cardiac patients. There is a markedly increased excretion of sodium and chloride. There is no change in phosphate, sulfate, or ammonia excretion or in nitrogen balance. There is a loss of extracellular fluid, a diminution in plasma volume and a loss of weight. The serum shows a decrease in chloride which is excreted in greater quantities than in the equivalent amount of body fluid lost.

Doubling the dose of mercurial approximately tripled the amount of fluid lost and doubled the duration of action. Comparison was made with metaphyllin injections (theophyllin-ethylene-diamine or aminophyllin) and with theobromine orally which caused a similar but much less sustained effect.

The effectiveness of the mercurials is influenced by ammonium chloride administration. The augmentation of diuresis obtained by giving ammonium chloride with a mercurial, is more than one would expect from the additive effects of the two when used separately. Also, one can depress the diuresis expected from a mercurial by giving beforehand sodium bicarbonate and producing an alkaline urine. A reasonable explanation of these augmentation and suppression phenomena is that in an acid urine the dissociation of mercury is greater and therefore more readily absorbed by the renal tubule. Ascorbic acid has recently been used in large quantities to augment mercurial diuresis and I suspect that any acid in large quantities may do the same.

The relative usefulness of the mercurials and the xanthines has been frequently discussed. The xanthines, both theobromine and theophyllin, are not as effective as the mercurials. However, the mercurials must be given parenterally for maximal effect whereas the xanthines can be given orally. A few deaths have been reported from the mercurials and there have been rashes, nausea and vomiting observed. Renal damage also may occur after their use. Ordinarily one can

give the xanthines in large doses for several days, although theophyllin in effective doses is likely to have undesirable side reactions. Renal damage from xanthines is not likely.

Dr. Harvey: Before we go on to the discussion, Dr. Andrus is going to talk briefly about some of the other techniques that are used in treating the patients with myocardial insufficiency.

Dr. Andrus: At the last session we paid particular attention to the early, perhaps primarily responsible, element in the production of congestive heart failure, which we described as myocardial inefficiency. We rehearsed the ability of digitalis to improve the work capacity and, by implication, the function of the myocardium. We mentioned at the time that physiological studies on man had not exonerated the myocardium from its share of responsibility in congestive failure, but had attracted attention to other abnormalities, particularly those of renal function. Dr. Newman has described the armamentarium which is available for the attack on that problem. I hope that he will, in discussion, speak more about the mechanism of the deficient renal function, particularly toward salt, insofar as it is understood, and the influence of diuretics on that.

The condition which is presented by the typical case of congestive heart failure, or "cardiac dropsy", is that of congestion, with a plethora of fluid, and a rather obstinate tendency to retain salt rather than to excrete it normally. I should simply like to mention certain factors which are regarded as important in the clinical management of cardiac failure in the hope of stimulating discussion.

In the first place, although it may be rather impertinent to stress it in this company, there is no therapeutic measure or drug available for the treatment of congestive failure which is a decent substitute for a thorough examination of the patient. The estimation at the beginning, and periodically, of the physical status of the individual is, of course, a primary essential. It is helpful and important to know from the patient's history two important points at least, the story of the progressive development of his disability and, very helpful, what he, himself, has found makes him most comfortable. These small details might seem unimportant but in planning the management of the case they may be very significant.

The first procedure should be rest. There has been a tendency

recently to deprecate the value of rest in the treatment of heart failure. It can be overdone but it cannot be discarded. It should be accomplished in the proper position, not with the patient flat and accumulating fluid in his lungs, but with his body and head raised or with his feet lowered so that if fluid accumulates it may accumulate in his extremities.

These patients are restless and often in great distress. Rest may be difficult to provide without most careful attention to details. A patient can exert himself more, and sometimes more harmfully, struggling with a bed pan than if he gets up to a handy commode. The individual should be assured a normal amount of sleep. Insofar as possible, emotional strains and worries should be relieved. Sedatives will be required and, in the early stages, the opiates are the most effective. In my experience, pantopon is somewhat less liable to produce nausea in these patients than morphine.

With regard to diet, in advanced congestive failure the hazard of the patient taking too much food is not likely to interfere. It is still useful to employ the Karell regime, which prescribes 800 cc of milk. That can be continued for a few days. The diet must be low in salt, because the deficient excretion of salt simply compounds the difficulty of relieving the dropsy. In recent years there has been a return of an early interest in very low salt diets—one to one and a half grams daily—with relatively unrestricted fluids. If such a diet can be made tolerable to the patient it is possible to avoid development of edema, to relieve symptoms considerably.

There are other obvious procedures, which would not escape attention but which are important to emphasize. If fluid has collected in the serous cavities, particularly in the pleural cavities, to remove that by thoracentesis is to confer additional breathing space almost cc for cc.

Additional measures are directed in the advanced cases to the reduction of auricular pressure. Raising the head and lowering the feet will foster a reduction of pressure in the right auricle. Constriction of the extremities by tourniquet is a temporary expedient, useful in acute failure, but it cannot be maintained. Venesection has a more prolonged effect.

The administration of oxygen must be tempered by consideration

of its purpose and its probable effectiveness. It is certainly an effective remedy. When necessary in acute failure it should be administered by mask. There is a good deal of evidence that a mask in which pressure or resistance can be opposed to expiration is more effective than a simple mask carrying on at atmospheric pressure. If oxygen must be administered over a period of days a mask becomes intolerable. Pure oxygen, unless somehow hydrated, becomes irritant. For protracted treatment an oxygen tent is the method of choice.

The therapy with digitalis I shall omit, since that was taken up last week, and Dr. Newman has discussed the use of diuretics. I shall only emphasize that they are more effective in the digitalized patient than in the undigitalized, and that the action of one superimposed upon that of the other may bring much more rapid and much more considerable relief.

Apropos Dr. Newman's historical reference to mercurials, I can't resist mentioning a preparation which is not used now, but which is older than the medical experience of most of us in this room—the Addison-Niemeyer pill which contained digitalis, squills and calomel.

I would like to express an additional word of caution at this point which refers back to my original statement that successive determinations of the physical status of the individual are essential. You can't start a routine on a patient in the state of congestive failure and go away and leave him. Dr. Newman has emphasized that you can't give ammonium chloride indefinitely. This is an outstanding example.

If treatment of the acute stages of congestive failure is effective, the problem then turns to maintenance therapy. This is, in fact, the planning, and testing, and management of a way of life for the patient calculated to maintain his circulatory efficiency at its highest level. Often it is possible for a person to carry on a useful and comfortable existence, even though he has had congestive heart failure. This requires maintenance quantities of digitalis leaf: at least one-tenth, at most two-tenths, gram; in terms of digitoxin two or three, rarely more, tenths of a milligram. It requires habits of rest calculated to diminish the constancy of the strain of existence. Exertion, particularly sudden or violent exertion, must be avoided if the hazards of increased circulatory load are to be escaped. The earliest guiding symptom is in most instances shortness of breath. The outstanding exception is the pa-

tient with aortic stenosis, whose symptoms of overdoing are more frequently those of forward insufficiency of the circulation, fatigue of the legs, general fatigue, fainting after exertion, or cardiac pain. After the patient has recovered, or after the therapeutic effect has been obtained, he often needs to be encouraged to undertake a comfortable degree of exertion. There are, of course, the unhappy cases in which it is not possible, by methods at our disposal, to restore the circulation to permit any useful activity. It is to be hoped that the future may hold a better understanding of circulatory deficiencies which will allow us to do for such patients more than we can do at this moment.

Dr. Harvey: The meeting is now open for questions and discussion. This is a very important subject, one with which everyone very soon begins to accumulate experience. As has been pointed out, there are no strict rules of thumb in handling patients with congestive heart failure and one accumulates skill by long experience; I think therefore that it might be interesting to ask some of the members of the staff here who have had considerable experience in this field to make comments and point out certain important features that have been brought out by the speakers.

Dr. Bordley: I would like to ask Dr. Newman to elaborate more on how salt restriction fits into diuretic therapy. It seems to me one of the most important methods of therapy.

Dr. Newman: In Marvin's series of cardiac patients, digitalis and salt restriction were sufficient treatment in 36 patients. Probably in the majority of cardiac patients in failure, diuretic drugs are not necessary to free the patient of edema. However edema can be eliminated more rapidly if diuretic drugs are used.

There is a defect in the "cardiac" kidney such that salt is not normally excreted. In a normal person, ingested excess of salt and water is rapidly excreted. In a normal individual on a restricted salt intake, the kidney will conserve salt to a marked degree. In the cardiac even with a large excess of salt and water in the body, an abnormally large fraction of ingested salt is retained; conservation of salt by the kidney is excessive. It has been postulated that cardiac failure results in forward failure in so far as the kidney is concerned and that the diminution in glomerular filtration results in a retention of salt and water. I think this is an interesting suggestion, but I don't think we can

account for edema on the basis of reduced glomerular filtration rate alone. The amount of salt appearing in the urine is the difference between filtration rate and tubular reabsorption. If the filtration rate is diminished, one might just as well blame the tubule for continuing to reabsorb so much salt. In chronic nephritis there is diminution in glomerular filtration, but there is frequently a failure to conserve salt due to decreased tubular reabsorption; in such a case we blame the tubule. In nephrosis there may be supernormal filtration, but there is edema due to excessive tubular reabsorption of salt. Thus in other diseases causing edema there is no direct correlation of filtration rate with salt retention. Tubular reabsorption is an equal if not a governing factor. These patients who have edema in spite of salt restriction and digitalis therapy require diuretic drugs. The most effective drugs are those which decrease the tubular reabsorption of salt;—the mercurials and xanthines, theobromine and theophyllin.

Water in large amounts has been used to treat edema. This practice is based on an old principle. By forcing water and causing a large diuresis one finds eventually that the output of water exceeds the intake. This results from the fact that the kidney is not a perfect organ. The kidney cannot excrete pure water. In other words the tubules cannot remove all the salt nor conserve all the salt in the glomerular filtrate. At a very high urine flow there is a small but definite continuous loss of salt. The composition of body fluid is then protected by losing water also. To make such a regime practical, it is necessary to give enormous quantities of water for several days.

There is certainly no sense in restricting fluid intake in edematous cardiac patients, because a low water intake will aid to some extent in the retention of salt. Restricting fluids means the tubule reabsorbs more water; with conservation of more water it is more likely to reabsorb salt. Fluids should be taken *ad lib.* or up to the point of the patient's tolerance. Restriction of salt not water is of primary importance.

Dr. Marshall: Is there any defect in the excretion of potassium by the kidney in cardiac failure?

Dr. Newman: From what I have observed there is no inability of the kidney to excrete potassium in cardiac failure, provided there is no primary renal damage.

Dr. Marshall: Why wouldn't it be good therapy to put potassium into a low salt diet to make it more palatable?

Dr. Newman: Actually we have done that. There is a preparation which has been recently marketed, a salt substitute, which is a mixture of potassium chloride, ammonium chloride and a few other salts that don't contain sodium. It doesn't taste exactly like table salt. However it makes the low salt diets more palatable to many patients and is certainly worth trying. Our dietitians have found it is quite satisfactory also for use in cooking some vegetables and meats.

Dr. Marshall: I would like to ask about giving mercurial diuretics every day if necessary or every other day on the assumption that the diuretic is all excreted in 24 hours. I was glad Dr. Andrus brought up the point of the Addison-Niemeyer pill because I have always felt that the action of the organic mercurials was by the same mechanism as the action of calomel. The effect is simply the initial stage of mercurial poisoning, which blocks the absorption of sodium chloride.

Dr. Newman: I agree that as far as we know we are giving a renal poison in a mild form.

Dr. Marshall: Would there be a definite limit as to how frequently it should be given?

Dr. Newman: That subject has recently been commented on by Gold. He suggests giving frequent "non-cumulative" doses.

Dr. Marshall: How long does it take the mercury from a single injection to be eliminated? What per cent comes out in 24 hours? Is there any such thing as a "non-cumulative" dose?

Dr. Newman: Many patients have been given mercurial injections once or twice a week for one or two years without any evidence of renal damage or other toxicity.

The question of how long the drug can be given would seem to depend on the individual patient's reaction. Certainly one should carefully observe the patient with urinalyses and tests of renal function. The problem of how frequently mercurials should be given and whether accumulation is dangerous is difficult to answer. It is our clinical impression that spacing the injections at twice weekly or weekly intervals gives satisfactory results. That accumulation of mercury in the kidney does occur has been demonstrated recently in dogs and patients. The kidney stores a much higher concentration of mercury

than other tissues analyzed and may retain significant amounts for at least a few weeks after discontinuing the administration.

Dr. Harvey: What about the time at which diuretics should be started in the patient with severe congestive failure. There seems to be a growing tendency to start diuretic drugs immediately with other treatment such as digitalis, rest and oxygen. I was of the opinion that diuretics given for congestive failure can actually be harmful if given at too early a stage in the treatment. I think Dr. Andrus answered this in part. He mentioned the fact that bed rest in the proper posture, diet, sedation, and digitalis are, after all, the most important things at the beginning. And perhaps the patient will do very well on that. It may be that renal circulation is so poor in the patient who comes in with untreated failure that diuretics aren't going to be effective.

Dr. Andrus: The circulatory effects of digitalis and the action of diuretics will be most fully complementary if diuretics are given after digitalization. It is worth remembering, too, that diuretics do not act immediately and their action persists for hours.

Dr. Newman: Certainly a patient should not be given mercupurin at 6:00 PM and be kept awake all night voiding.

Dr. Marshall: I would like to add a word about the use of diuretics in the patient who has diminished renal function due to nephritis. We frequently wish to use diuretics in these cases and frequently fear to use them because of possible damage to the patient. I wonder, has anyone had experience with this?

Dr. Harvey: Can you answer that, Dr. Newman?

Dr. Newman: I think that it has been definitely shown that in active nephritis the acid salts and mercurials result, not always, but usually, in a flare-up of the nephritis with more cells, casts and albumin in the urine, and further depression of function. In a patient with healed nephritis or nephrosclerosis I believe these drugs are relatively safe, but one should proceed cautiously and observe the patient closely. In a patient who is likely to have renal irritation, it is wise to give a small first dose of mercurial slowly. A small test dose has been recommended routinely in all patients by some authorities because of the rare occurrence of other serious toxic reactions. Apparently the xanthine diuretics do not cause significant renal damage even in active nephritis.

While we have at our disposal quite effective methods for the treatment of edema due to cardiac failure, there is much room for improvement in our knowledge and application of these methods and for new drugs. There is need for diuretic drugs like the xanthines causing loss of sodium chloride, but having a greater and longer action with less undesirable side effects. New methods for administration of the mercurials and for augmenting their effect may be developed.

The reasons for the successes or the too frequent failures in therapy can be found only by close clinical observation of the individual patient and by the use of physiological studies and principles.

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DIFFUSE CONGENITAL CYSTIC HYPERPLASIA OF STOMACH CLINICALLY SIMULATING CARCINOMA*

Report of a Case

H. WILLIAM SCOTT, Jr., and TORRENCE P. B. PAYNE

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Exclusive of peptic ulcer, benign lesions of the stomach simulating carcinoma are relatively uncommon. It has been estimated that less than 0.5 per cent of gastric tumors are benign (1). Among non-neoplastic lesions, chronic gastritis, syphilis, and tuberculosis occasionally have been known to resemble gastric cancer. We have recently encountered a case of diffuse cyst formation involving the mucosa and submucosa of the stomach which clinically simulated gastric carcinoma.

Case Report

J. M. (408483), a 58 year old colored laborer, was first admitted to the Urological Service of the Johns Hopkins Hospital on January 13, 1947, because of suspected left renal tumor. Family history was irrelevant. Past history showed gonorrhea at age 19 and scarlet fever at 21; otherwise there had been no significant illness and the patient had been well and active until the onset of the present illness.

Two years before admission the patient developed the first of a series of episodes of dull, aching periumbilical pain radiating to the left lower quadrant. These pains had no relation to meals and were of an intermittent character, occurring every two to three months and persisting for three to four days at a time. There was no associated nausea or vomiting. In the six months prior to admission he had poor appetite and lost about 18 pounds in weight. During this time he had occasional epigastric pains from which he obtained some relief by taking bicarbonate of soda. He had become constipated and had been bothered by hemorrhoids for several months. Six weeks before admission he had developed burning on urination in addition to the other complaints. Because of a suggestive filling defect in the upper pole of the left kidney, found on intravenous pyelography in his dispensary studies, he was admitted to the Urological Service. Retrograde pyelograms were then performed and were entirely normal. There was microscopic hematuria and pyuria which was thought to be on the basis of chronic prostatitis. A gastro-intestinal series showed a peculiar filling defect in the antrum of the stomach, and the patient was transferred to the surgical service.

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Physical Examination. T. 98.6, P. 96, R. 20, B.P. 118/75. The patient was a thin, emaciated, elderly colored man who was partially edentulous. Nose and throat showed no abnormalities and there was no lymphadenopathy. The lungs were clear and the heart was within normal limits. The abdomen was scaphoid, non-tender, and there were no palpable masses. Rectal examination showed external hemorrhoids and a small firm prostate.

Laboratory Studies. Hemoglobin was 12 gms. per cent; W.B.C. 5,200 with normal differential. Urine contained a few WBC and an occasional RBC per high power field but was otherwise negative. P.S.P. showed 76 per cent excretion in 2 hours. N.P.N. was 33 mgm. per cent; Chloride 98 milli-equivalents/liter; CO_2 combining power 30 milli-equivalents/liter. Serological test for syphilis was negative. Gastric analysis showed a total acid of 12 clinical units and no free acid. Stools were guaiac negative. X-ray of the chest revealed moderate emphysema, but was otherwise not remarkable. Cholecystograms and barium enema showed no abnormalities. Gastro-intestinal series showed a large rounded lesion in the anterior wall of the stomach in the antral region (Fig. 1). There was no ulceration of the mucosa over this lesion. There were many small areas of thickening of the gastric wall adjacent and extending some distance from the rounded shadow. Peristalsis was impaired by these changes, but the stomach emptied fairly well within 5 hours. An infiltrating carcinoma of the stomach was felt to be the most likely diagnosis.

Operation (*H.W.S., Jr.*): Under ether anesthesia, the abdomen was entered through an upper abdominal midline incision. There was no free peritoneal fluid. Abdominal exploration revealed no metastases in the liver, which was normal in appearance. There were no gross abnormalities of the large or small bowel. Examination of the stomach showed it to be moderately dilated but the gastric wall was normal in external appearance with no visible evidence of ulcer or tumor. On palpation there was a diffuse rubbery thickening of the gastric wall extending from the pylorus to the mid-portion of the body of the stomach. There was a sharp line of demarcation at the pylorus, and the duodenum was normal in appearance and quite mobile. The thickened gastric wall was not indurated, and there was a fairly sharp upper border at about the mid-portion of the body of the stomach above which the texture of the gastric wall seemed normal. A biopsy was taken of the full thickness of the wall of the stomach in the antral region. Frozen section showed no evidence of carcinoma. On extending the biopsy opening, the stomach wall was found to be filled with small cysts ranging up to the size of a pea and filled with watery mucoid fluid. The mucosa was not inflamed or ulcerated, but the gastric lumen in the antral region was not much larger in caliber than the operator's index finger.

Since the thickened mucosa tended to obstruct the emptying of the stomach and since we could not be positive that we were dealing with a benign lesion, we decided to do a partial gastrectomy. The greater and lesser curvatures of the stomach were then mobilized and the distal two thirds of the stomach were re-

sected. It appeared grossly that we were transecting the stomach at least 3 inches proximal to the upper border of the hypertrophied gastric wall. An anticolic gastrojejunostomy of the Polya type was then constructed and the abdomen closed.

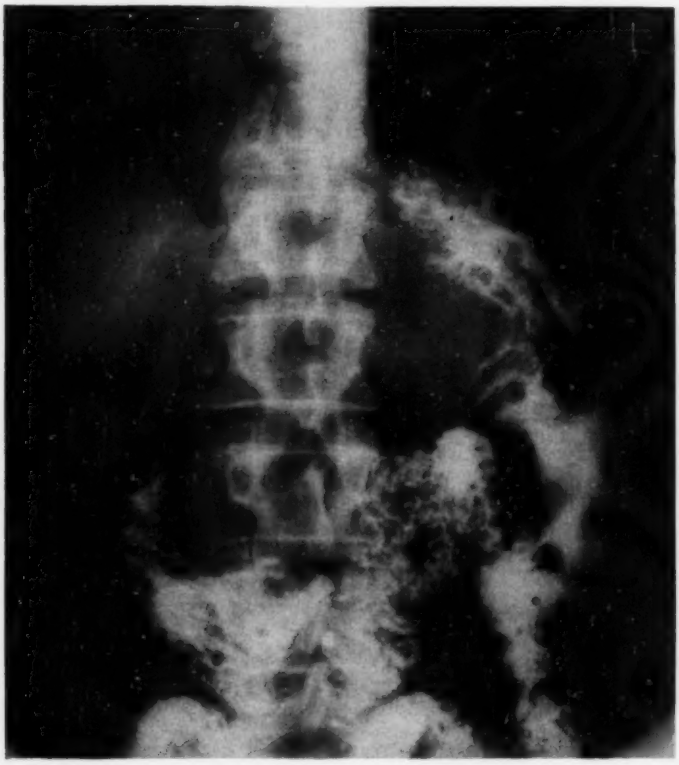

FIG. 1. GASTRO-INTESTINAL SERIES. NOTE FILLING DEFECT IN ANTRAL REGION

Course: After operation the patient was maintained on gastric suction for 24 hours and parenteral fluids were given for 3 days. Graduated oral feedings were taken well and convalescence was entirely uneventful. Postoperative gastrointestinal series showed a satisfactory stoma with no retention. The patient was discharged from the hospital on the 16th postoperative day. In the 5 months since discharge he has been followed in the G.I. clinic and has been maintained on

a full soft diet with intermediate feedings. He has gained 12 pounds in weight, has remained entirely asymptomatic, and is back at his accustomed work.

Pathological Study (*T.P.B.P.*)

Gross Description: The specimen (S.P. 47-218) consists of the distal 15 cms. of the stomach. The serosa is smooth and pink. Along the greater curvature in the prepyloric region there is a fresh linear incision measuring about 5 cms. in

FIG. 2. Distal end of resected stomach. Diffuse cystic change in submucosa Small abscess in submucosa with surrounding scarring. Continuity between epithelium of cysts and surface epithelium of mucosa (A).

length and having six black silk sutures in it; in this region the wall of the stomach feels thicker than elsewhere. By extending this incision the stomach is opened along its greater curvature. The mucosa does not have the usual rugose appearance but is lifted up into somewhat thicker folds; one of these folds along the greater curvature in the pyloric region is especially prominent. No ulcers or erosions of the mucosa are seen. The gastric wall is thicker than usual, and in the

area of the previously described incision measures about 1 cm. in thickness. In this area several cystic spaces, measuring about 5 mm. or less in greatest diameter, are seen lying beneath the mucosa. On careful examination small cystic spaces are seen scattered throughout the entire wall of the stomach. Representative blocks are taken for microscopic study.

Microscopic Description: In all of the sections there are cystic spaces of varying size lined by columnar or cuboidal epithelium and situated in the mucosa or sub-

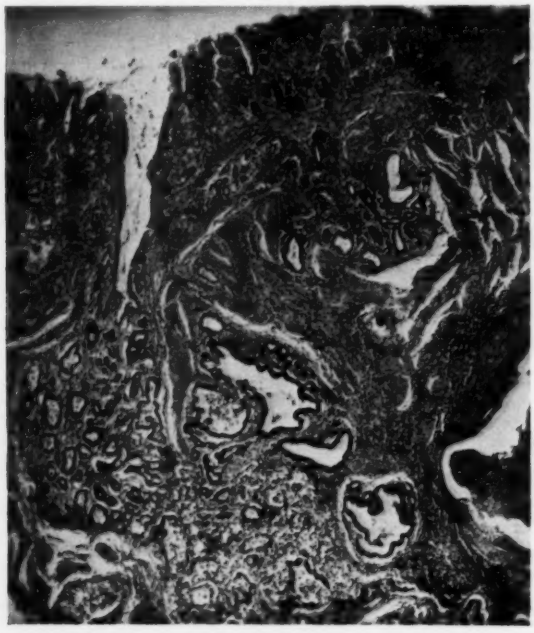

FIG. 3. HIGHER POWER OF (A) FIGURE 2. NOTE GLANDULAR OUTPOUCHINGS AROUND CYSTS

mucosa. In some areas definite continuity can be traced between the epithelium of the cysts and the surface epithelium of the mucosa (Figs. 2 and 3). In several of the larger cysts there is intracystic papillary formation (Fig. 4). Small glandular outpouchings, lying in a myxomatous stroma, arise from others (Fig. 2 and 3). Many of these larger cysts have a delicate layer of smooth muscle around them, as if they had not completely penetrated the muscularis mucosae, while others are quite definitely in the submucosa. In all of the sections this diffuse cystic change

is seen and it is clear that the process must have extended beyond the limits of the resected stomach. There is no inflammation or scarring (Fig. 5) except in the sections taken from the thickened area in the prepyloric region of the stomach. Here in the submucosa a small abscess is seen (Fig. 2). It is apparently formed within one of the larger cystic cavities, for fragments of intact epithelium are still present around the periphery where, in addition, there is early fibroblastic prolifera-

FIG. 4. DISTAL END OF RESECTED STOMACH. CYSTS WITH PAPILLARY FORMATION. EDGE OF ABSCESS ON RIGHT

tion. Among the numerous polymorphonuclear leucocytes, bacterial stains reveal no definite organisms, but several gram positive fragments are seen which may represent the relics of digested bacteria. Collections of acute inflammatory cells are also seen in several adjoining cystic spaces, and the surrounding stroma is infiltrated with them (Figs. 2, 3 and 4). In addition, there is definite scarring of the submucosa in this region. In none of the sections is any erosion of the mucosal epithelium seen.

Final Diagnosis: Congenital cystic hyperplasia with abscess formation in stomach.

Comment: While the most interesting features of this case are the cystic changes in the mucosa and submucosa of the stomach, there was a striking similarity between the clinical aspects and those of gastric carcinoma. In retrospect, it is difficult to establish any signi-

FIG. 5. PROXIMAL END OF RESECTED STOMACH. DIFFUSE CYSTIC CHANGE IN SUBMUCOSA. THERE IS NO INFLAMMATION OR SCARRING HERE

ficant points of differentiation. In an elderly man with anorexia, weight loss, epigastric pain, gastric anacidity, and a filling defect in the gastric antrum on roentgen examination, a diagnosis of carcinoma should be made. It is unfortunate that preoperative gastroscopy was not performed in this patient, but, regardless of what it might have disclosed, exploration would doubtless have been necessary. Even at operation there was considerable doubt as to the benign nature of the lesion.

Pathologically there was no evidence of any neoplastic process and the diffuse cystic change in the mucosa and submucosa was, as far as we are aware, unlike any previously described lesion (2). In chronic follicular gastritis there may be epithelial hyperplasia with cyst formation in the gastric mucosa (3). Cystic changes are also occasionally seen in the edges of chronic peptic ulcers. In this case, however, there was no evidence of any previous inflammatory process or ulceration. The cystic change was diffuse with definite papillary formation and probably involved the whole stomach. It was only in the antrum that there was any evidence of inflammation and this was relatively recent, well localized, and undoubtedly represented infection occurring secondarily in one or several of the cysts. This probably resulted in the more recent symptoms.

This condition appears to represent a developmental defect in the formation of the gastric glands which was present since birth. As far as is known there was no associated cystic disease in lungs, liver, pancreas or kidneys.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal)

An Integrated Practice of Medicine. By HAROLD THOMAS HYMAN. Illus. 4131 pp. $50.00 W. B. Saunders Company, Philadelphia, Pennsylvania, 1946.

This "Integrated Practice of Medicine" was written by a group of practitioners expressly for the guidance of the general practitioner. It is an extensive work consisting of four volumes and a separate comprehensive index. The ambitious aim of the senior author was to place in the hands of the general practitioner a single authoritative reference source devoted not only to the subject matter of internal medicine but of all the associated fields of general medicine, and a survey of the preclinical sciences in addition. Among others, this broad scope includes such diversified topics as psychiatry, dentistry, obstetrics, emergency major surgery, rehabilitation, immunology, chemistry, radiology, dietetics, and therapeutics. The plan of the author was to integrate the data of this complex material as they pertain to the specific complaints presented by the patient to the general practitioner in order that he might become "the compleat physician". To facilitate the correct interpretation of symptoms and signs, 318 tables of differential diagnosis have been prepared.

The magnitude of such an undertaking is manifest, and one marvels at the authors' intrepidity. To fulfill its purpose, such a work would need to be comprehensive yet adequately detailed, entirely up to date, and thoroughly sound in its opinions. Although possessing these attributes to a considerable degree, this work unfortunately possesses none of them to a sufficient degree. The authors have endeavored to encompass too much in too small a space, employing a format which does not permit additions to the text as new developments appear. Thus Loeffler's syndrome is disposed of in four lines as an acute pneumonitis occurring frequently in patients with vasomotor rhinitis and bronchial asthma. Only four lines are devoted to folic acid, no mention being made of its use in the treatment of sprue. The newer anti-malarial agents are not discussed. In speaking of the toxicity of streptomycin, reference is not made to the vestibular reactions which may occur, and the statement is made that "toxicity is negligible and bacterial fastness unusual". In discussing the treatment of acute rheumatic fever, it is related that "vitamin P obtained from red pepper and lemon peel is said to control the disease", and "there is abundant evidence to suggest that the salicylates inhibit the antigen-antibody reaction that is responsible for the vascular and connective tissue injury in acute rheumatic fever". But four lines are devoted to the ditary treatment of disturbances of the circulation, there being no discussion of the advantages of salt restriction. Many would disagree that "only a small pro-

portion of the patients with congestive heart failure with sinus rhythm is favorably influenced" by digitalis. In considering the treatment of subacute bacterial endocarditis with penicillin, the use of heparin is advised without reservation, it being said that "... nor have there been untoward consequences as the result of the increase in bleeding time". Not all would agree that "true lobar pneumonia a relatively uncommon disease" or that "the present drug of choice is sulfadiazine" in its treatment. A single line is devoted to rheumatic pneumonia in which the impression is given that the entity occurs only "in a small number of the most severely ill patients". More recent experiences with anti-biotics already requires rewriting of the sections devoted to the treatment of lung abscess and empyema, which are described as being amenable only to surgery. In a table of "Confirmatory Diagnostic Aids in Respiratory Infections", a sputum culture is advised when tularemia or psittacosis is suspected, and the Weil Felix reaction is recommended for Q fever. The cold agglutinins test is not listed for virus pneumonia.

It is the opinion of the reviewer that the general practitioner would find a more abundant and stimulating source of information and help in the classical texts devoted to medicine, pharmacology, physiology, and clinical biochemistry than is contained in these four volumes.

P. A. T.

Anesthesia in General Practice. By STUART C. CULLEN. Illus. 260 pp. $3.50. The Yearbook Publishers. Chicago, Illinois, 1946.

This short volume is a fitting companion to the handbook series. As is proper, the emphasis is upon the practical aspects of anaesthetic practice and not on theoretical considerations. In every sense it is a manual directed to medical students and those others of the profession whose interest in anaesthesia is general. The text is presented in a simple, straightforward manner. In addition to the usual discussions of agents and technique, oxygen therapy and pre- and post-anaesthetic management are discussed. There is a short but adequate discussion of curare by the author who is also one of the outstanding authorities on the clinical application of this drug in anaesthesia.

The book is characterized by a most sensible attitude toward the subject. The material is up-to-date. An otherwise soporific subject is relieved by some lively cartoons.

M. H. H.

The Diagnosis and Treatment of Bronchial Asthma. By LESLIE N. GAY. Illus. 334 pp. $5.00. Williams & Wilkins Company, Baltimore, Maryland, 1946.

A book must not be read or criticized before the preface is carefully scanned. The author makes it very clear that except for the chapter on Physiology of Respiration the contents of the remaining chapters represent a written record of his experiences, impressions, opinions and advice. For those of us who have been closely associated with Dr. Gay, the reading of this book produces all the pleasant sensations that enshroud nostalgia.

It is quite true that the treatment of pollens as causes of asthma smacks of provincialism and therefore the internist and allergist west of the Mississippi River will be left hungry for information. Nevertheless, the treatise was not intended to be the usual type of textbook, and such a criticism is unjust. The same reasoning may be withheld in view of the author's "narrow-mindedness" in regard to food hypersensitivity. The brevity of his discussions on food allergy and its place in the etiology of asthma parallels exactly his personal attitude in regard to the smallness of its importance.

The chapter on the Pathology of Asthma is tedious to read, with all the case reports and autopsy findings. But still, the information contained in the many reports represents a world of information to be appreciated to its fullest by "students". This section of the book will long outlive the author.

The section on treatment is complete in every respect. Though the discussions on helium might well have been abridged, still the recording of interesting information is always pleasing, and the picture of the development of helium that is written gives one a solid footing for its appreciation. If the reader cares nothing for background, however, he will become impatient with the author and with his resourcefulness and thoroughness for detail.

The book is everything that it is intended to be, and for those who respect the author and wish to have his counsel, they will find in his work complete satisfaction.

E. L. K.

Essentials of Endocrinology. By ARTHUR GROLLMAN. 2nd Edition. Illus. 644 pp. $10.00. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.

In 1941 the first edition of Grollman's *Essentials of Endocrinology* was published. Concepts concerning many endocrine disorders had been based upon fanciful and ill-founded conjectures rather than experimentally established, scientific facts. The author attempted to approach the clinical aspect of the subject from a sound scientific background. To do this, it was necessary to review and critically evaluate a vast amount of literature and to correlate this into an orderly exposition of the anatomy and functions of the endocrine glands. On the whole the results were splendid and the conclusions sound.

In the past six years there has continued to be a great deal of experimental investigation in endocrine physiology, although the basic concepts have not been materially altered. Of even greater importance, there has been an increased application of newer methods of study and hormone assay to the problems of clinical diagnosis. In the second edition the author has introduced a considerable amount of the new material. Of note are a chapter of 23 pages on "The Hypothalamus and Its Disorders" and discussions of exophthalmic ophthalmoplegia, thiouracil therapy and alloxan diabetes.

A number of Grollman's conclusions are still open to question. He states that the hypothalamus influences gonadal function not by direct action of the hypophysis but rather by nervous impulses to the gonad itself. Likewise, it is stated

that the available evidence indicates that a single compound is responsible for all of the activities of the normal adrenal cortex and that only in pathologic disorders is androgen produced. The various corticosterones and androgens which have been isolated from the adrenal or from the urine of castrates are believed to be artefacts or end metabolites. In the section on sex differentiation ambisexual potentials and genetic mosaics are well discussed, but the reader is confused by the statement on p. 449 that "in the human, intersexes are always genetically female" and on p. 451 that the sex aberration which occurs most frequently is male pseudohermaphroditism in which the abnormal individual is genetically a male.

For the student of endocrinology the writer has done an excellent service in presenting in one volume a well-organized and clearly written compilation and analysis of basic facts, documented with fairly abundant references. From the standpoint of the clinician one regrets that the author does not make a greater effort to point out the application of the newer methods of study to the differential diagnosis of the etiology of various symptom complexes such as sexual precocity and sexual infantilism. For instance, as carried out in most laboratories, the follicle-stimulating-hormone assay has served to differentiate accurately pituitary infantilism from primary hypogonadism. The writer states that the test is of little practical value. It is true that the F.S.H. assay as ordinarily performed does not distinguish between different types of gonadotrophin and that the 17-ketosteroid excretion does not measure precisely androgenic activity. Nevertheless these tests are important tools in the armamentarium of the endocrinologist and have been of aid in clinical diagnosis. The writer does not emphasize sufficiently the fact that many symptom complexes may be caused by a lesion either of the hypothalamus, in the pituitary or in one of the "target glands". There is need for a text which will guide the clinician in the methods of study to determine the site of the lesion. In a book which is otherwise so complete one is surprised that there is no adequate description of some of the clearcut but recently described disorders such as the syndrome of ovarian agenesis and dwarfism, and that associated with the primary hyaline degeneration of seminiferous tubules. One misses a discussion of the general metabolic effects of the androgens. Although he is correct in divorcing clinical diagnosis from the fanciful concepts of the past, it is to be regretted that so able a writer as Grollman does not make clearer the application of modern methods of study in the differential diagnosis of endocrine disorders.

L. W.

Gynecology Including Female Urology. By LAWRENCE R. WHARTON. 2nd Ed. Illus. 1027 pp. $10.00. W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.

As stated in the preface, the second edition of Wharton's *Gynecology Including Female Urology* incorporates a number of revisions and additions which serve to increase the usefulness of this already valuable text and bring it up to date along those lines in which the most recent advances have been made.

In that portion of the book devoted to gynecology, notable changes are to be

found in the two chapters on embryology and congenital malformations. These have been rewritten and enlarged to include such subjects as sex differentiation in early embryonic life and the recent studies on sexual infantilism resulting from agenesis of follicular elements in the ovary. Likewise, the chapter dealing with disorders of ovulation and ovarian function now includes a discussion of the significance and clinical importance of changes in the basal temperature curve associated with ovulation.

The phenomenon of precocious menstruation, its causes and treatment, is dealt with at some length, and is a new and interesting addition to the chapter devoted to the general subject of menstruation.

The section on female urology has been improved by some rearrangement of the subject matter and the addition of two new chapters. One of these describes the technique of water cystoscopy, the various uses of different types of water cystoscopes, and the application of this technique as a valuable adjunct or alternate to the Kelly air method in the study and treatment of urinary conditions in women. The other new chapter, devoted to the female urethra, properly emphasizes the clinical importance of the anatomy, structure, and relationships of this unit in considering the various abnormalities, diseases, and injuries to which it is subject.

In dealing with the problem of the surgical removal of ureteral stones, the author has amplified his original description of the technique of ureterotomy in the upper middle, or lower thirds of the ureter, and supplemented the text with excellent illustrations.

At the end of the book, two important chapters have been added, one dealing with chemotherapy and the other with irradiation and its application to gynecology and female urology. The first of these is a clear and concise resumé of the present state of our knowledge concerning the actions, uses, and toxic manifestations of the sulfonamides and mandelic acid, followed by an equally good discussion of penicillin and other antibiotic agents. The second combines very logically the generally accepted views concerning the proper use of x-ray and radium in dealing with various gynecological conditions and with tumors of the urinary tract. Controversial points are brought out and complications following irradiation are freely discussed.

These and other revisions serve to retain the position of this book as a leading authoritative text which may be read with profit by student and practitioner alike.

E. H. R., Jr.

Techniques and Procedures of Anesthesia. By JOHN ADRIANI. Illus. 404 pp. $6.00. Charles C. Thomas, Springfield, Ill. 1947.

In a technique reminiscent of his popular "Pharmacology of Anesthesia," Dr. Adriani has again employed the outline form to good advantage. Though not so profusely illustrated as the former volume, it is not quite so sparing of words in the actual text. Comments, reasons, advantages and disadvantages of the subjects under discussion are set down in an orderly 1, 2, 3, fashion. In this way the author achieves a remarkable clarity of text and eliminates the dross of words

that make technical texts difficult. The coverage of the material is complete, all forms of anaesthesia are dealt with, inhalational, regional, rectal intravascular. In addition resuscitation and oxygen therapy are briefly surveyed. Again in this volume, Dr. Adriani draws attention to the mechanical equipment, machines, tanks, valves, etc., a subject too often neglected.

This book will be useful to those actively engaged in either learning or teaching anaesthesia. For the former, its clarity will introduce some order into steps of technique and for the latter it will provide an organized outline useful for teaching. It is however, "cook-book" anaesthesia and will only be a way station for those seriously interested in the subject. The illustrations and tables are satisfactory. The main virtue of this book lies in the severe clarity and orderliness of the material presented.

M. H. H.

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Advances in Internal Medicine. Vol. 2. Editors: WILLIAM DOCK and I. SNAPPER. Illus. 642 pp. $9.50. *Interscience Publishers, Inc., New York, New York, 1947.*

Advances in Pediatrics. Vol. 2. Editors: S. Z. LEVINE, ALLAN M. BUTLER, L. EMMETT HOLT, JR., and A. ASHLEY WEECH. Illus. 407 pp. $6.75. *Interscience Publishers, Inc., New York, New York, 1947.*

American Illustrated Medical Dictionary, The. By W. A. NEWMAN DORLAND. 21st ed. Illus. 1660 pp. $8.50. *W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.*

American Sanatorium Association, a Brief Historical Sketch. By LEWIS J. MOORMAN. Illus. 72 pp. $1.00. *National Tuberculosis Association, New York, 1947.*

Atlas of Cardiovascular Diseases. By IRVING J. TREIGER. Illus. 180 pp. $10.00. *The C. V. Mosby Company, St. Louis, Missouri, 1947.*

Bone and Bones: Fundamentals of Bone Biology. By JOSEPH P. WEINMANN and HARRY SICHER. Illus. 464 pp. $6.00. *The C. V. Mosby Company, St. Louis, Missouri, 1947.*

Cambridge Medical History. By SIR WALTER LANGDON-BROWN. 119 pp. *Cambridge University Press, New York, New York, 1947.*

Clinical Radiology. A Correlation of Clinical and Roentgenological Findings. Edited by GEORGE UTLEY PILLMORE. 2 vols. 1600 pp. Illus. $45.00. *F. A. Davis Company, Philadelphia, Pennsylvania, 1946.*

History of the American Medical Association, 1847–1947. By MORRIS FISHBEIN. Illus. 1226 pp. $10.00. *W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.*

Infant Feeding with Evaporated Milk. Illus. 40 pp. *Evaporated Milk Association, Chicago, Illinois, 1947.*

Infant Nutrition: A Textbook of Infant Feeding. By P. C. JEANS and WILLIAMS MCKIM MARRIOTT. 4th ed. 516 pp. $6.50. *C. V. Mosby Company, St. Louis, Missouri.*

Insides Out. By JOHN MASON BROWN. Illus. 202 pp. $2.00. *Whittlesey House, McGraw-Hill Book Company, Inc., New York, New York, 1947.*

Internal Medicine in General Practice. By ROBERT PRATT MCCOMBS. 2nd ed. Illus. 741 pp. $8.00. *W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.*

Medical Writings of Anonymus Londinensis. By W. H. S. JONES. 168 pp. $2.75. *Cambridge, at the University Press; The Macmillan Company, New York, New York, 1947.*

Methods of Diagnosis. By LOGAN CLENDENING and EDWARD H. HASHINGER. Illus. 868 pp. $12.50. *The C. V. Mosby Company, St. Louis, Missouri, 1947.*

New and Nonofficial Remedies: Issued Under the Direction and Supervision of the Council on Pharmacy and Chemistry of the American Medical Association. 749 pp. $3.00. *J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.*

Nursing. By LULU K. WOLF. Illus. 534 pp. $3.50. *D. Appleton-Century Company, New York, New York, 1947.*

Osteotomy of the Long Bones. By HENRY MILCH. Illus. 294 pp. $6.75. *Charles C. Thomas, Springfield, Illinois, 1947.*

Practical Emulsions. By H. BENNETT. Illus. 568 pp. $8.50. *Chemical Publishing Company, Brooklyn, New York, 1947.*

Preoperative and Postoperative Care. By WILLIAM J. TOURISH and FREDERICK B. WAGNER, JR. Illus. 338 pp. $6.00. *F. A. Davis Company, Philadelphia, Pennsylvania, 1947.*

Principles and Practice of Medicine, The. By HENRY A. CHRISTIAN. 16th ed. 1539 pp. $10.00. *D. Appleton-Century Company, Inc., New York, New York, 1947.*

Signs and Symptoms. By CYRIL M. MACBRYDE. Illus. 439 pp. $12.00. *J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.*

Textbook of Physiology. By WILLIAM D. ZOETHOUT and W. W. TUTTLE. 9th ed. Illus. 723 pp. $4.75. *The C. V. Mosby Company, St. Louis, Missouri, 1946.*

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